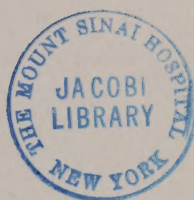







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## Mesotheliomas in Hamsters Following Intrapleural Injection of Asbestos

W. E. SMITH, M.D., LLONAS MILLER, Ph.D., J. CHURG, M.D.,  
AND I. J. SELIKOFF, M.D.

*Madison, New Jersey*

Exposure to asbestos dusts has been associated with pulmonary carcinoma and with mesothelioma of the pleura and peritoneum in man (1, 2). Conversely, malignant tumors have not been reported after inhalation or intratracheal injection of numerous preparations of asbestos in a variety of species of animals (3, 4) with exception of lesions found in two mice (5). Literature on experimental studies of lung cancer has been reviewed (6). Recently, Wagner (7) reported mesotheliomas in rats after intrapleural injection of chrysotile and crocidolite varieties of asbestos.

In hamsters, we found extensive pleural fibrosis with development of pleural mesothelioma (8) after intrapleural injection of the amosite variety of asbestos. Additional mesotheliomas have now appeared in hamsters after intrapleural injection of amosite or chrysotile, and the results are summarized in the present paper.

### METHODS

Samples of soft chrysotile, harsh chrysotile and amosite were processed to average fiber lengths of 67, 36 and 18 microns, respectively, by a method developed in this laboratory (9). Each of 45 male hamsters was given a single right intrapleural injection of 25 mg of one or another of these preparations suspended in 0.5 cc of 0.9 per cent sodium chloride solution. There were 15 animals in each treatment group. A fourth group of 15 hamsters was set aside as an untreated control. All groups were maintained on a pelleted feed, but 1 per cent amosite was baked into the daily ration of the group that had received amosite intrapleurally, and 1 per cent soft chrysotile was baked into the daily ration of the groups that had received intrapleural injection of the chrysotile samples. The animals were maintained on these diets until they died or were sacrificed. At necropsy, tissues and organs were described and examined histologically.

### FINDINGS

Pleural adhesions involving the right lung and often extending into the pericardial sac and left pleural cavity were found in 27 of 37 hamsters that

From the Health Research Institute, Fairleigh Dickinson University, Madison, N. J. This work was supported by Public Health Service Research Grant CA 07681 from the National Cancer Institute.

died or were sacrificed from the 97th to the 563rd day after start of test. In hamsters without such adhesions, deposits of asbestos were found in the subcutaneous tissue of the chest wall, the injection having evidently failed to reach the pleural space. Extensive pleural and pericardial adhesions were found in hamsters treated with each of the three varieties of asbestos tested. Asbestos fibers were numerous in these adhesions, and there were occasional asbestos bodies. There was marked pleural thickening and calcification. In specimens removed early in the experiment, adhesions consisted of granulomatous inflammation. Multinucleated giant cells, often filled with asbestos fibers, then became a prominent feature. In later specimens, cellular infiltrate was sparse and the adhesions consisted principally of fibrous tissue. In each of the

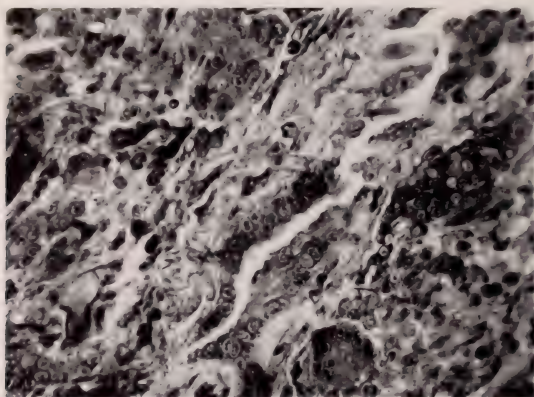


FIG. 1. Pleural adhesion with epithelial-like cells lining a cleft, 255 days after intrapleural injection of soft chrysotile asbestos fibers. (440  $\times$ )

three treatment groups, pleural adhesions were found that contained islands of epithelial-like cells that often lined narrow clefts (Fig. 1). The adhesions ranged up to 4 mm in thickness. In occasional animals, nodular masses up to 10 mm in diameter were found between the right lower lobe and the diaphragm, with ill-defined edges blending into the adhesions. These nodular masses simulated tumors, but microscopically were found to consist of necrotic tissue and calcification surrounded by granulomatous inflammation or fibrosis.

Very different were the findings in four hamsters in which the chest was largely filled with solid masses of tumor tissue. These tumors compressed the lungs and lay between them and the chest wall (Fig. 2). The first was found in a hamster sacrificed because of labored respiration 244 days after injection of amosite. It was a 40 x 20 x 15 mm mass that nearly filled the thorax and extended through the diaphragm into the abdomen. It was composed of plump,

pleomorphic polygonal cells (Fig. 3) and some areas of spindle cells (Fig. 4). Numerous metastases composed of spindle cells were found in the liver (Fig. 5).

On the 358th day, another of the amosite-treated hamsters was sacrificed because of severe ascites due to fluid containing tumor cells. All lobes of the right lung were encased by dense yellow adhesions, but on the diaphragmatic

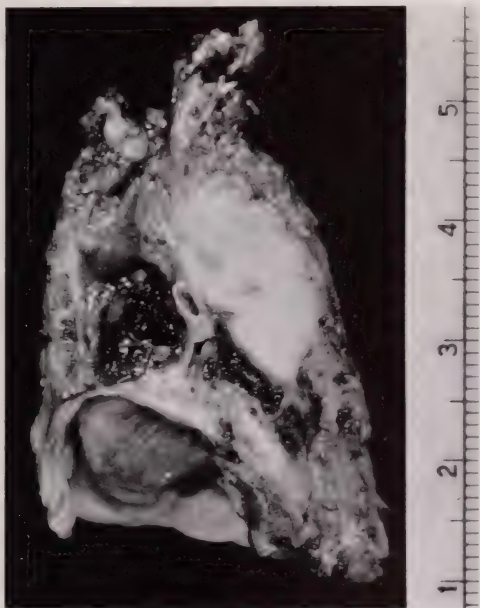


FIG. 2. Sagittal section of the thorax of a hamster showing a large intrapleural tumor (white) compressing right lung and displacing heart anteriorly. The upper surface of the compressed right lower lobe is bound to the tumor. The base of this lobe is bound to the diaphragm by dense fibrous adhesions. Similar adhesions bind the base of the heart to the diaphragm. 527 days after intrapleural injection of harsh chrysotile asbestos fibers.

aspect of the right lower lobe there was a solid mass of grayish white tumor tissue 25 mm in diameter which had penetrated the diaphragm. It consisted of irregularly shaped polygonal, sometimes elongated cells. There were at least 50 nodules of such tumor tissue 1 to 11 mm in diameter in the mesentery. Metastases were found in lymph nodes, lungs, thymus, liver and kidney.

In the group treated with harsh chrysotile, mesotheliomas were found in two hamsters, one examined on the 419th day and the other on the 527th day. In



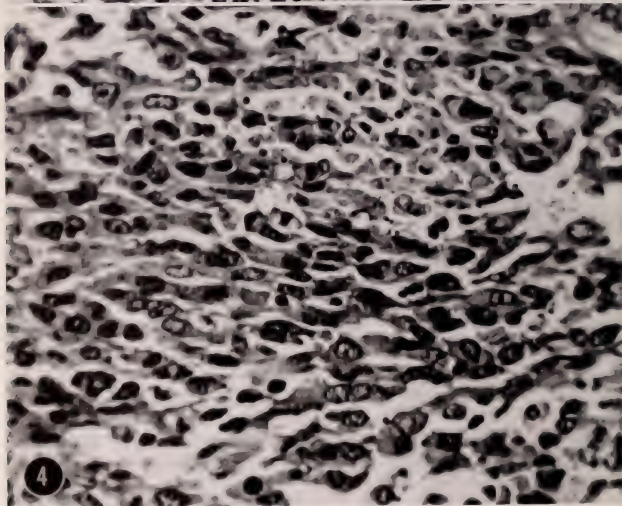
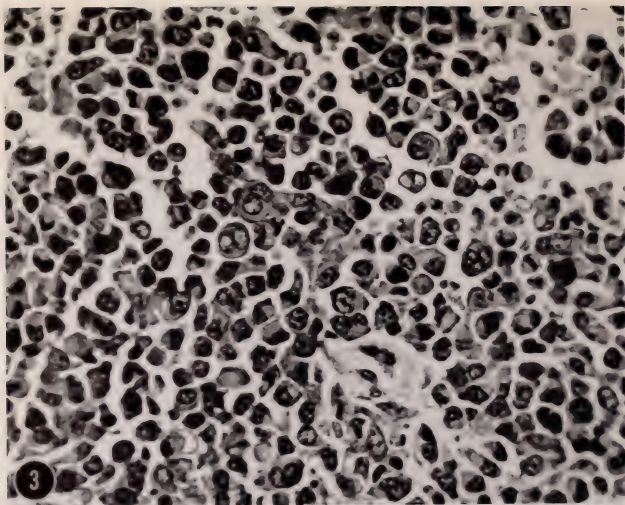


FIG. 3. Mesothelioma, epithelial cell type. Pleomorphic polygonal cells. 244 days after intrapleural injection of amosite asbestos fibers. (550  $\times$ )

FIG. 4. Same tumor as Figure 3. Area of spindle cells. (550  $\times$ )

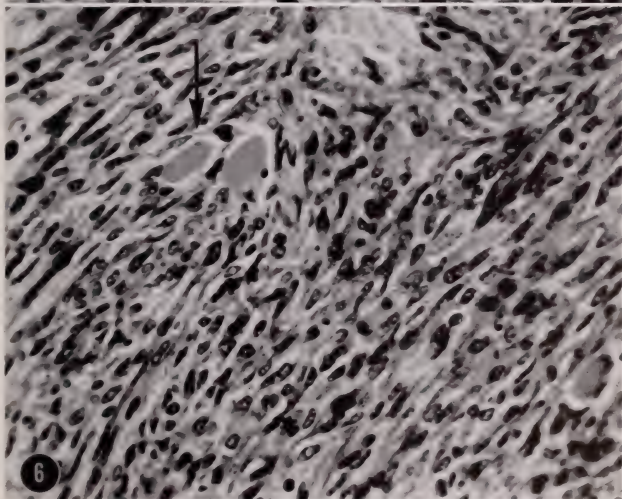
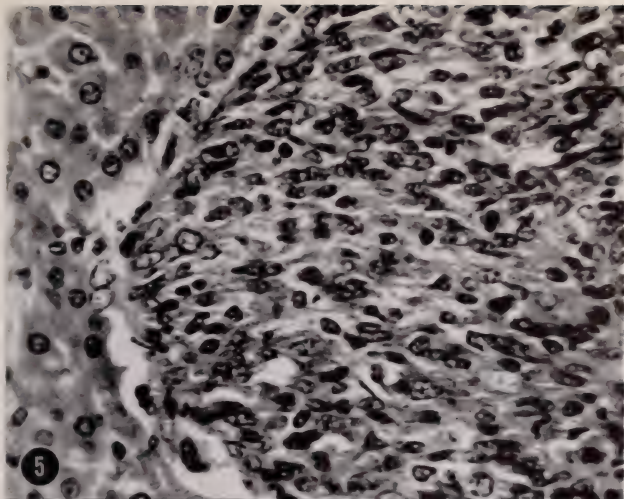


FIG. 5. Metastasis in liver from tumor illustrated in Figures 3 and 4. Metastasis consists mainly of spindle cells. (550  $\times$ )

FIG. 6. Mesothelioma, spindle cell type, infiltrating chest. Note remnants of muscle fibers (arrow). Tumor demonstrated in the gross in Figure 2. (550  $\times$ )

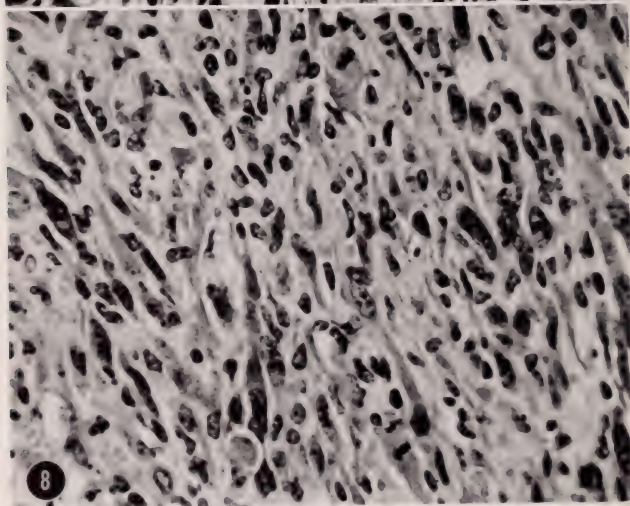
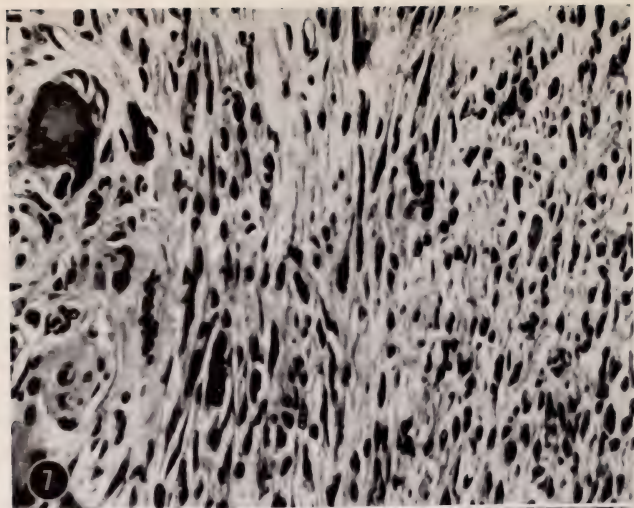


FIG. 7. Early mesothelioma. Granuloma with foreign body giant cells at left and spindle cell tumor to right, 482 days after intrapleural injection of amosite asbestos fibers. (550  $\times$ )

FIG. 8. Same tumor as Figure 7, showing cell pleomorphism and mitoses. (550  $\times$ )



the first of these, sacrificed because of paralysis of both hind legs, a 30 x 20 x 15 mm solid tumor lay between the right lung, chest wall and vertebral column. It consisted of densely packed, basophilic, elongated cells. No metastases were found. Pieces of this tumor were transplanted into the right posterior thigh muscles of 6 young adult hamsters. The transplanted tissue grew progressively. Ten weeks after transplantation, solid tumor masses 50 to 70 mm in diameter had developed at the transplant sites in each of these new hosts. Also in each of these new hosts, metastases were found in the lungs. The cells of the transplanted tumor, and of its metastases, resembled those of the original tumor.

In the original group of hamsters, an animal observed to have labored respiration on the 527th day after intrapleural injection of harsh chrysotile was sacrificed on that day. There was a solid, fine textured, hard, white tumor lying between the heart and the vertebral column and displacing the lungs downward (Fig. 2). The lungs were bound to the chest wall and diaphragm by adhesions or tumor. This tumor was composed of spindle cells that infiltrated the chest wall (Fig. 6).

In addition to the four massive tumors mentioned above, a small tumor was found in a pleural adhesion of a hamster examined on the 482nd day after injection of amosite. The border of this tumor and the granulomatous tissue of the adhesions is shown in Figure 7. A more anaplastic area of the tumor is illustrated in Figure 8.

No tumors were found in the pleural space of hamsters treated with the long-fiber preparation of soft chrysotile, but islands of epithelial-like cells were prominent in pleural adhesions in this group (Fig. 1). In one animal of this group, there was a firm spherical mass, 11 mm in diameter, in the right axilla. This proved to be a fibrosarcoma enclosing a deposit of asbestos fibers.

No tumors were found in the untreated control group.

Histological studies of the gastrointestinal tracts from the animals of all groups have not yet been completed. In each group, several animals still survive.

#### DISCUSSION

The location of the five tumors between the lung and chest wall resembled the location of tumors described as pleural mesotheliomas in man (10). One of the large tumors found in amosite-treated hamsters resembled tumors described as the epithelial type of mesothelioma in man, another resembled the mixed epithelial and fibrous type. The neoplastic nature of these two hamster tumors was attested by invasive growth and distant metastases. The two large tumors found in hamsters treated with harsh chrysotile, and the small tumor in one of the amosite-treated animals, resembled tumors described as the fibrous type of mesothelioma in man. The neoplastic nature of one of these hamster tumors was demonstrated by successful transplantation to new hosts. Islands of epithelial-like cells, sometimes lining narrow clefts, were found in pleural adhesions of hamsters in which a diagnosis of tumor was not made. These islands are of interest as possible precursors of tubular types of mesotheliomas.

Further experimental studies on effects of asbestos in animals are reported elsewhere (11, 12).

#### CONCLUSIONS

The golden Syrian hamster is a suitable species for investigation of biological effects of asbestos.

Pleural mesotheliomas were induced in hamsters by two varieties of asbestos. Evidence is presented for the neoplastic nature of these tumors.

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# Meningioma of the Falx-Tentorial Angle with Successful Removal: A Case Report

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Meningiomas of the falx-tentorial angle—*carrefour-falco-tentorial* (CFT) (1)—are rare. Surgical removal of tumors in this area is difficult and relatively few successful removals have been accomplished. The following case with its interesting clinical, radiographic, and operative features is reported.

## CASE REPORT

The patient, a 58 year old, right-handed female was admitted to The Mount Sinai Hospital on November 23, 1961. She complained of a staggering gait of two weeks' duration. The patient had noted decreased hearing in the right ear one year previously. The hearing loss, associated with tinnitus, had been progressive. Two episodes of vertigo had occurred. Two weeks prior to admission the patient noted that she was unsteady and would stagger to the right. She had noted some weakness and diminished dexterity of the right hand; however she had been able to continue work as a machine operator.

## Examination

The gait was slow, shuffling, and unsteady; there was a tendency to fall to the right. Turning to the right was more difficult than turning to the left. A left central facial weakness and a left homonymous hemiamblyopia was present. There was no nystagmus. However, normal optokinetic nystagmus was absent. The right corneal reflex was diminished and there was variable sensory loss in the distribution of the right trigeminal nerve. Air conduction and bone conduction were diminished in the right ear. Fine movements of the left hand were impaired; there was a left hemisensory defect to double simultaneous stimulation. The reflexes were within normal limits. There was no finger-to-nose ataxia. Ice water calories revealed no response in the right ear but a prompt response in the left ear. The Bekeasy audiogram showed no recruitment and no tone decay.

The electroencephalogram done on admission was normal. However, the postoperative brain wave test showed bilateral cerebral dysfunction accentuated in the right posterior cerebral region. The cerebrospinal fluid protein was 59 mg%.

The pneumoencephalogram revealed the lateral ventricles to be somewhat enlarged. The pineal gland was calcified and displaced forward and to the right. The posterior part of the third ventricle was markedly displaced forward. The fourth ventricle was depressed downward and slightly anteriorly. The aque-

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duct was not seen. The lateral autotomogram (Fig. 1) demonstrated these features. Other roentgenograms (not presented) disclosed in the region of the falx crescentic air shadows which outlined a large, almost spherical, mass. The air shadow under the tentorium appeared cut off in its anterior half, probably outlining, thus, the posterior margin of the tumor. The air shadow in the cistern of the velum interpositum was displaced upward and forward. There was no air in the right cerebello-pontine angle.

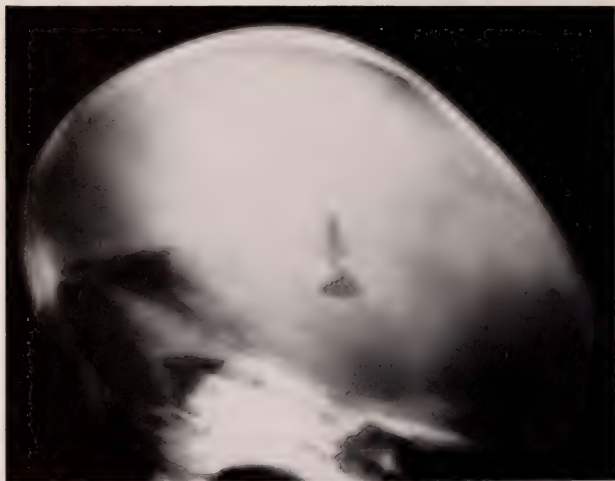


FIG. 1. Pneumoencephalogram (lateral autotomogram) reveals downward displacement of the fourth ventricle. The posterior portion of the third ventricle is visualized and is markedly displaced anteriorly. These structures surround the meningioma arising from the tentorium at the falx-tentorial angle.

The right carotid angiogram revealed the anterior cerebral artery to be in the midline. There was a rather wide sweep of this artery in the lateral view. The middle cerebral artery was slightly displaced laterally. The posterior part of the anterior choroidal artery was slightly anteriorly displaced. In the venous phase, there was marked elevation of the posterior part of the internal cerebral vein. Running from the posterior end of the internal cerebral vein of Galen there was a large vein traversing the falx and draining into the superior sagittal sinus. The straight sinus was only faintly visualized. The connection between the vein of Galen and the anterior end of the straight sinus was not seen. These findings suggested a blockage of either the anterior part of the straight sinus or the posterior part of the vein of Galen with collateral circulation be-

tween the internal cerebral vein and the superior sagittal sinus through the dural sinus of the falx.

A right vertebral angiogram showed the basilar artery to be pushed forward and in close contact with the clivus. The medial and lateral posterior choroidal arteries were displaced forward. The posterior cerebral arteries were dis-

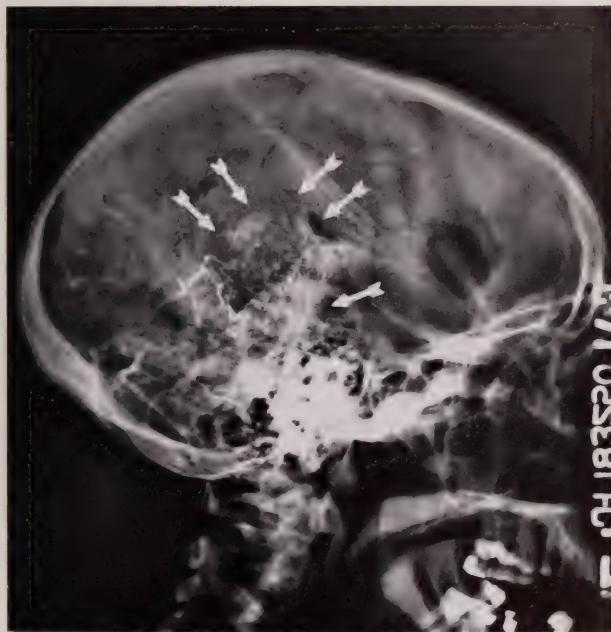


FIG. 2. Right vertebral angiogram. The meningeoma stain is clearly visualized. Arrows surround the tumor. Compare this study with the pneumoencephalogram.

placed laterally. The midportions of the superior cerebellar arteries were depressed downward. There was a large meningeal artery running in the midline along the posterior aspect of the posterior fossa and through the falx-tentorial angle supplying blood to a large tumor located in the posterior portion of the tentorial notch. This large, staining mass measured 13 cm in height and 10 cm in length (Fig. 2). The straight sinus was not visualized. The roentgenologic impression was a meningeoma arising from the falx-tentorial angle.

### *Operation*

The first operation was carried out on December 11, 1961. Under endotracheal anesthesia and hypothermia, the right ventricle was cannulated via a right frontal burr hole and the fluid was noted to be under increased pressure. A right reverse Frazier flap was turned. A low bone flap was turned to expose the occipital and parietal lobe. The occipital lobe was partially resected and retracted. A firm, non-suckable, vascular meningioma was found at the falx-tentorial angle extending through the incisura. The tentorium was divided and the tumor partially herniated from the posterior fossa. The tumor was biopsied since removal appeared to be a formidable procedure.

### *Course*

Following operation the patient made a slow recovery. A left homonymous hemianopsia was present. Her gait improved. She was discharged to a convalescent home on January 15, 1962.

Following discharge the patient did well as a housewife until May 1962. At that time progressive gait difficulty, progressive recent memory defect, trouble feeding and dressing herself, and, finally, urinary incontinence occurred. The patient was placed in a nursing home. She was readmitted for evaluation on July 29, 1962. Physical examination at that time revealed fullness of the right occipital-parietal flap. Neurological examination revealed that the patient was unable to walk without support. She was alert but disoriented as to the day of the month. The patient acknowledged her mentation defects. She was slow to understand and execute commands. Right-left disorientation was present. A dense left homonymous hemianopsia and mild left facial weakness were present. The right corneal reflex was absent. Air and bone conduction were diminished in the right ear. Upward gaze was impaired; there was no nystagmus. Power in the extremities was good. Mild hyperreflexia was present. The superficial abdominal reflexes were absent. The toes did not move on plantar stimulation. There was a left hemihypesthesia to pin prick as well as position sense errors in the left toes. There was dystereognosis of the left hand. There was no gross finger-to-nose ataxia.

Pneumoencephalogram demonstrated marked enlargement of the lateral and third ventricles. The posterior third ventricle and pineal were displaced forward. The aqueduct and fourth ventricle were displaced forward and down. The fourth ventricle was moderately enlarged. The medial wall of the right atrium was indented by a large midline mass which was located in the posterior portion of the falx-tentorial notch. The vertebral angiogram was essentially unchanged from previous examination.

### *Second Operation*

After full evaluation, a total removal of the tumor was planned to reverse the inexorable progression of symptoms. Steroids were begun the day prior to surgery. On August 16, 1962, endotracheal anesthesia was begun and the patient was placed on the hypothermia blanket. The temperature dropped

from 92°F to 83.5°F during surgical removal of the tumor. A catheter was placed in the right frontal horn and the bone flap was re-elevated. Bleeding from the posterior portion of the longitudinal sinus was controlled with silver clips and sutures. The tumor appeared to be larger than at the operative procedure nine months previously. A wedge of the tentorium was removed. After freeing the superior surface of the tumor, the capsule of the tumor was opened and the contents were removed with curets, suction, and the electric loop. There was considerable, but never uncontrollable, bleeding. Finally the capsule and remaining tumor were removed. The site of origin from the undersurface of the tentorium was partially excised. A small nubbins of tumor was removed from the superior surface of the tentorium and the base was charred. The procedure lasted five hours; the patient required four units of blood. Thirty grams of tumor were sent to the neuropathology laboratory.

#### *Course*

During the first 30 hours following surgery the patient responded in the usual fashion. She moved all extremities though paresis of the left upper extremity was present. Nystagmus and bilateral Babinski responses were noted. She said "yes" on the evening of the operative procedure. Thirty-two hours after surgery, the patient suddenly coughed and was momentarily apneic. The patient then became lethargic and respiratory difficulty with suprasternal retraction commenced. Caffeine sodium benzoate, 500 mg, was given intravenously with some improvement. The patient replied verbally and moved all extremities on command. The ventricular catheter was opened but no fluid refluxed. A tracheotomy was performed one hour after the onset of respiratory difficulty with improvement of respirations. Because of her semicomatose condition, 90 grams of urea (as a 30 per cent solution in 10 per cent invert sugar) was given intravenously. Slow improvement occurred in the next 48 hours. Steroids were discontinued 4 days after surgery because dark brown material (guaiac positive) was aspirated from the nasogastric tube.

One week after operation the patient was alert and oriented to date and person. Progressive improvement continued despite persistence of fever and a disconcertingly large subgaleal fluid collection. By October 29, 1962, the day of discharge, the patient was vastly improved. She was able to walk by herself. She was able to dress herself and was not incontinent. She was fully oriented to date and was aware of current events. Serial subtraction was well done. Her handwriting was legible. Full upward gaze was present. The left visual field defect was unchanged. However, the left hemisensory defect was improved.

She was hospitalized at a convalescent home for three months and then returned to her home. When last seen, April 8, 1963, her mental condition was normal. She kept her own home and did her own cooking and laundry. She shopped in the company of her daughter. She was free of headache. Examination revealed a cautious but not ataxic gait. She was able to walk without assistance. The left visual field defect remained. The fundi were normal. Hearing

was improving in the right ear. Neurological examination was otherwise unremarkable.

### *Pathological Report*

Sections showed a highly cellular meningioma in which the cells were uniform, fusiform in shape, and contained large, moderately staining nuclei. The nuclei varied in shape; some were oval and others were moderately elongated. The cytoplasm was eosinophilic and fibrous with a poorly defined cell membrane. The cells were arranged in broad sheets or intersecting columns. A thin, dense, fibrous connective tissue membrane was attached to the tumor.

### DISCUSSION

Meningiomas of the pineal region and posterior part of the third ventricle may arise from the falx-tentorial angle (CFT) or the velum interpositum (VI) of the third ventricle. A total of 27 cases have been reviewed in the literature (2-5). Of these cases 22 were operated upon. In 16 cases the tumor was approached directly with an attempt at total removal. Of these cases 10 had a successful outcome.

In two successfully operated cases of CFT meningioma, Olivecrona approached the tumor via a parieto-occipital exposure (6). The tentorium was split about one centimeter from the straight sinus. In the first case the tumor stretched through the tentorial notch to the dorsal surface of the cerebellum. It was removed by suction. In the second case the splenium of the corpus callosum was split. After this a firm, vascular, gray-yellow, well-defined tumor was encountered under the choroid plexus of the third ventricle. It extended to the left. A large vein coursing across the tumor (? internal cerebral vein) was ligated. Upon removal of the tumor, the impression of the quadrigeminal plate could be seen.

Heppner (7) reported two successful cases in children (velum interpositum origin). One tumor was approached via an occipital flap. The occipital pole was retracted and the tentorium split one centimeter lateral to the straight sinus. A tumor mass was found immediately beneath the tentorium and removed piecemeal. The main part of the tumor was adjacent to the quadrigeminal plate. It pushed the choroid plexus of the third ventricle forward. There were bilateral expansions into the ambient cisterns. The branches from the posterior choroidal and anterior superior cerebellar arteries which supplied the tumor were clipped. In the other case, the tumor was totally removed by a parietal transventricular approach. The tumor was adherent to the velum interpositum.

Araki (8) reported two cases which did well following surgery. Both tumors were approached by splitting the corpus callosum. The first patient died 15 months after surgery. Horrax removed a 40 gram tumor overlying the corpora quadrigemina (9). The tumor was exposed by occipital lobectomy. Poppen removed a meningioma arising from the incisura (CFT) through a right occipital



bone flap, resecting the right occipital lobe (5). The patient died from a pulmonary embolus three months after discharge.

One successful case was done by Olivecrona (CFT meningioma) by a combined infratentorial and supratentorial approach (2). Rozier removed a meningioma by the infratentorial approach (4). This tumor presented in the superior vermis and separated the cerebellar hemispheres. It arose from the tentorium, most likely in the CFT region.

There were six unsuccessful cases. Three were reported by Castellano and Ruggiero (2). In these cases, the initial approach was infratentorial. In two cases, additional tumor was removed later by the supratentorial approach. Castellano and Ruggiero discussed the operative features of tentorial meningiomas (2). Four advantages of the supratentorial approach were listed:

1. It affords better visualization.
2. It facilitates resection of the infiltrated portion of the tentorium.
3. Early section of the tentorium around the site of attachment reduces bleeding.
4. In cases of perforating meningioma it permits total extirpation in one stage.

They discuss two of the above cases in which the patient underwent suboccipital craniectomy. In each case the operation was difficult. In one case the patient had a stormy postoperative course and was left in poor physical condition with marked ataxia of the right hand. In another case, respiratory arrest followed the suboccipital approach. In this patient, a parietal-occipital flap was turned the following day and the residual tumor removed. However, the patient expired on the fourth postoperative day. Considering the difficulties encountered with the suboccipital (infratentorial) approach, they deem the supratentorial approach indispensable in CFT (falx-tentorial angle) meningiomas.

Heppner (6) reported two unsuccessful cases of CFT meningioma in which the supratentorial approach was utilized. The first was a 21 year old male who expired three days after surgery. Pathological examination revealed that the left internal cerebral vein had been clipped. The second was a 2 year old child who expired postoperatively.

Barrows and Harter (10) reported a 47 year old female who underwent occipital craniotomy under hypothermia. Ventricular fibrillation occurred during the procedure and the patient expired. Autopsy revealed a meningioma in the incisural notch arising from the tentorium.

#### SUMMARY

A case of successful removal of a CFT (falx-tentorial angle) meningioma is reported. This represents the eleventh case in the literature in which a meningioma was successfully removed from the region of the pineal and posterior part of the third ventricle. The operative cases from the literature are briefly reviewed.

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## Pancreatitis and Its Complications: Combined G.I. Surgical Conferences

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The Combined G.I. Surgical Conference on "Pancreatitis and Its Complications" convened at The Mount Sinai Hospital, New York, N.Y., Clinical Amphitheater at ten-thirty A.M., Dr. L. Burrows, presiding.

*Dr. Burrows:* We decided to have a conference today on pancreatitis and its complications. Unfortunately, on the surgical wards at Mount Sinai, we do not see as many cases of pancreatitis as they do at the city hospitals, but now that we are affiliated with Greenpoint, we see many cases of pancreatitis there.

Now, I do not know whether we see them there because of the different economic groups that we service at Greenpoint or because we have Dr. Dreiling as our Chief there; but, nevertheless, they do turn up at Greenpoint, and in the last month or so we have had some fascinating cases appear on the medical and surgical wards of that hospital. To begin, we are going to have Dr. Feldman present a few of these cases.

*Dr. Feldman:* The first case is Mrs. K. She was a 37 year old alcoholic who was admitted on November 30, 1963, with a four-day history of acute epigastric and left upper quadrant pain radiating to the back. This was associated with anemia and fever. X-rays of the chest revealed an elevated left diaphragm and blunting of the left costophrenic angle. The admission flat film of the abdomen disclosed pancreatic calcification, an associated sentinel loop; and thus was suggestive of acute pancreatitis.

In the past, the patient gave a history of having three previous similar episodes ten years prior to her present admission at Greenpoint.

On physical examination, the patient weighed 75 pounds. She had a mass in her left upper quadrant which was very tender. She had a markedly elevated white count. Serial amylase determinations revealed an elevation above 500 units and in addition, she exhibited an increased fasting blood sugar.

A diagnosis of acute pancreatitis was made and therapy included nonoperative management in which the patient was given antibiotics, intravenous fluids, Levin tube and administration of blood.

Serial G.I. series defined the mass in her left upper quadrant and its decrease in size. The fever resolved within the first ten days of hospitalization. The tenderness decreased and the mass was not palpable after the second week.

Her serum amylase was followed serially. The amylase remained elevated above the 600 range and her sedimentation rate was consistently in the 70 range. The patient gained 10 pounds while in the hospital over a 2½ month period. Her clinical condition improved markedly.

*Dr. Burrows:* Before we show the patient's x-rays, I want to apologize to

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the G.I. group. We could not obtain these x-rays from Greenpoint Hospital until late last evening, and Dr. Frankel, at very short notice, has consented to read the films today.

*Dr. A. Frankel:* In lieu of a radiologist, I will just briefly point out the radiographic documentation. These are selected plates from three separate G.I. series which demonstrate a large extrinsic filling defect on the fundus of the stomach. These films were taken on the third of December and show this large defect in the lateral projection.

The film that Dr. Feldman just put up demonstrates calcification presumably in the pancreas. A comparable lateral film taken several weeks later suggests a slight decrease in the size of the mass. Here on January 15th, in a slightly different projection which is difficult to compare, we see this defect which is notably smaller. The radiologists at Greenpoint, who have reviewed all the projections, feel that this represents approximately a 40 to 50 per cent reduction in the volume of a presumed pseudocyst projecting on the posterior wall of the stomach.

*Dr. Feldman:* The second case of pancreatitis has as its etiology the association of gallbladder disease rather than alcoholism.

This is a 79 year old diabetic male who entered on December 9, 1963, with an 18 hour history of generalized abdominal pain associated with nausea, vomiting and diarrhea. There was no previous history of jaundice or fatty food intolerance or previous G.I. disturbance.

Following admission, serial amylase determinations revealed an initial serum amylase of 147 and at four-hour intervals, 300, 700, and then 500. In addition, on x-ray, there was elevation of the diaphragm and a vague suggestion of pancreatic calcification. His abdomen was markedly tender, especially in the right upper quadrant. He also was managed conservatively, with Levin tube drainage, intravenous fluids, and antibiotics. There was subsidence of tenderness in the right upper quadrant and decrease in serum amylase values.

A secretin test on this patient revealed an elevated icteric index with a normal pancreatic function. An oral cholangiogram, taken after the subsidence of his acute abdominal pain, revealed nonvisualization of the gallbladder and common duct. An I.V. cholangiogram revealed a normal visualization of the common ducts with nonvisualization of the gallbladder.

*Dr. Frankel:* These films briefly represent what has just been described.

This film represents a double dose oral cholecystogram plus an I.V. cholangiogram, and these films are representative tomographic cuts showing a relatively normal common duct with nonvisualization of the gallbladder.

*Dr. Feldman:* The final case is a 68 year old Polish female who was admitted November 30, 1963, with a one-week history of acute onset of abdominal pain associated with nausea and vomiting. There was no previous history of jaundice or fatty food intolerance.

On physical examination, the liver was firm, smooth and three fingerbreadths down. A mass was noted in the epigastrium which was fixed and nonpulsatile.

The admission white count was 20,000 with a shift to the left. She exhibited

an elevated sedimentation rate and serum amylase determinations were always above 200.

A calcified mass was noted in the region of the gallbladder on routine abdominal films. Nonoperative management, including Levin tube, antibiotics and nothing by mouth, did not result in any improvement.

On the twenty-fifth hospital day, abdominal exploration revealed a mass in the lesser sac. During the dissection, pus was encountered in a pancreatic abscess which was drained.

Liver biopsy taken at the time of surgery showed periportal fibrosis. Culture of the purulent material revealed *Pseudomonas* and *Staphylococcus aureus*.

Postoperatively, serial sinograms were used to delineate the size of the abscess cavity. A secretin test revealed normal pancreatic study and a depressed icteric index.

*Question:* Did this tract continue to drain pus?

*Dr. Feldman:* Yes. Minimal amounts.

*Dr. Frankel:* A G.I. series shows flattening of the widened sweep of the duodenum in its medial aspect. There is a suggestion of displacement of the antral portion of the stomach by an extrinsic mass. No pancreatic calcification is seen, but a rim of calcification is seen in the gallbladder. There is a mushroom catheter in the abscess cavity through which contrast media was introduced. The contrast media readily passes into the G.I. tract, outlining the fundus of the stomach, the antral region and the sweep of the duodenum. There is, therefore, a communication between the abscess cavity and the stomach or duodenum.

*Dr. Burrows:* I want to thank Dr. Frankel again. He did this on very short notice.

We, therefore, have three cases that represent a spectrum of the many cases of pancreatitis from Greenpoint. Two of these are associated presumably with cholecystitis and one with alcoholism. Earlier this year we had an occasion to make a diagnosis of pancreatitis in the middle of the night, when we could not obtain an amylase either from Greenpoint or Mount Sinai for technical reasons. This particular patient appeared with abdominal pain, distention and generalized tenderness. We went back to the past literature, in which Otto Loewe described the adrenalin-mydratic test. I do not know if everybody is familiar with it. In the suspected pancreatitis case, you take a solution of 1:1000 adrenalin and drop it into one conjunctival sac. If ipsilateral dilatation ensues, you can assume a diagnosis of pancreatitis. We corroborated our clinical diagnosis on that basis, and it was proven correct the next day when the amylase came back over 1,000.

Dr. Dreiling, would you like to discuss this disease entity?

*Dr. D. A. Dreiling:* These three cases certainly represent an interesting point of departure in the problem of handling acute and chronic pancreatitis.

It is certainly true that we see a large number of patients with pancreatitis at Greenpoint Hospital, and I should like to presume that the diagnosis is made simply because of clinical familiarity.

I would like to make a few comments now on the individual cases before

launching into a very brief re-evaluation of our modern methods of handling acute and chronic pancreatitis.

The first patient, Mrs. K., represents the typical picture that we see in the alcoholic pancreatitis. This patient developed calcinosis. She has had periods in the past in which she has had steatorrhea, although she did not present with steatorrhea probably because she was on a high alcohol and low food intake diet. Her secretin test is extremely interesting. I think the figures were something like 3.0 cc per kilogram and 35 millicivalents per liter, showing a tremendous depression bicarbonate and practically no enzymes. This is the end stage of destruction by pancreatic inflammation. She had normal gall-bladder function. This case, therefore, is not associated with biliary tract disease.

Her present episode developed a complication of pseudocyst. I think one of the most important reasons to present such a case is to illustrate that these pseudocysts will subside and that not every patient with a pseudocyst should be subjected to immediate cyst decompression. Unless the pseudocyst is expanding or the clinical course of the patient is deteriorating, these patients should be treated as patients with acute pancreatitis—starved for a long period of time. Again, she demonstrates what inadequate therapy will do, because she was put on such a regimen and improved. Then she was fed, and every time she was fed she developed an exacerbation.

At present, she has shown evidence that her pancreatitis is diminishing. The pancreatic pseudocyst is becoming much smaller, and we propose to send her home for a period of six weeks and then have her return.

The problem is: "Is she going to stop drinking?" If she does not stop drinking, this pseudocyst is not going to disappear and we will probably have to institute internal drainage. Also, with the assistance of alcoholic intake, she will go on to other complications.

Thus, the natural course of these pseudocysts is a tendency to subside. How do they subside? Probably by eroding back into the obstructed duct which was the basis of the pancreatitis in the first place. This drainage back may be rather rapid. Dr. Richman and I had a case a number of years ago in which a huge pseudocyst, which was the size of a large coconut, disappeared within 48 hours. On the other hand, it may be much slower, as in this case, demonstrating that there is probably an extensively diseased duct with multiple areas of stenosis.

The second patient, Mr. S., demonstrates the occurrence of acute pancreatitis as a complication of biliary tract disease. Here, again, I think the secretin test is of some interest because it was done about two weeks following the acute attack. He, too, pulled out his Levin tube and discontinued his starvation therapy and developed an exacerbation.

The secretin test showed absolutely normal function. The findings were 4.2 cc H<sub>2</sub>O, 110 mEq L and 36 U/kg of enzyme, indicating that this type of pancreatitis represents a mild complication of the biliary tract disease. There is a rapid tendency of the pancreatic function, like the pathology in the gland,

to recover. At the time we did the secretin test, he had a biliary flow curve indicating a nonfunctioning gallbladder. This gallbladder dysfunction was then confirmed by the inability of the gallbladder to be visualized by oral cholecystography or by intravenous cholangiography.

Biliary tract associated pancreatitis should be treated by eradication of biliary tract disease. In this elderly gentleman there was some debate as to whether or not this should be done. I think that one attack of acute pancreatitis and an exacerbation of pancreatitis is sufficient warning that this patient is in danger. There was some discussion on the rounds at Greenpoint as to whether or not the common duct should be explored at the time of surgery. If we accept the etiology of biliary tract disease associated pancreatitis to be mechanistic, we imply that in some way there is an obstruction to both the biliary tract and to the pancreatic duct system. I think the time that one does the initial surgery for eradication of biliary tract disease, one should explore the common duct. If one finds pathology, one must eradicate it. If one does not find pathology, I would not advocate the addition of sphincterotomy.

The third patient, Mrs. Z., is another patient with biliary tract disease who, after all, shows an unusual complication. It is rare for the biliary tract disease associated pancreatitis to go on either to pseudocyst formation or to abscess formation. This is one patient who demonstrated both.

When she came in with her x-ray findings, the diagnosis was missed completely. She had a history of pain for several weeks but no clear-cut biliary tract disease history and no history of biliary tract colic could be elicited. The initial impression was that of carcinoma. We were agreeably surprised when at the time of operation, the pancreatin tumefaction did not feel like carcinoma. It felt a little soft. Judicious further exploration landed us into this huge abscess cavity.

At the time of operation we recognized that she, too, had biliary tract disease. She had stones and a contracted, thick-walled, gallbladder. We could not do anything at the time but drain this abscess. A biopsy was taken of the abscess wall, which should always be done in any pancreatic tumor. The report was acute and chronic inflammation. We are, therefore, certain that we are dealing with a pancreatic abscess. The rest of the pancreas looked normal.

The fascinating additional facet of this case is what has followed. I think we could almost have suspected it all along before these sinograms were taken, because here is a woman in whom we evacuated a huge pancreatic abscess and in whom postoperatively, only minimal drainage was obtained externally. We know that when we drain this type of case, a pancreatic fistula develops because of disruption of the ducts entering into the abscess cavity. The usual course is for pus to drain for several days and then one would expect the development of a pancreatic fistula. She did not behave in this manner.

The tendency in acute pancreatitis or pancreatitis in general is to form fistulae when the pancreas is manipulated. But there is also the very interesting tendency of these fistulae to drain back into the G.I. tract. This was originally observed in patients who had Whipple operations with hemitranssection of the



pancreas. In this case, we have visual demonstration that internal fistulization has occurred. Whether the secretions are into the stomach or duodenum is not clear. The last impression of the Department of Roentgenology at Greenpoint Hospital was that it was draining into both places.

I would like now to show some other evidence that internal fistulization occurs. The first slide that we have is a picture of a pancreatic abscess which ruptured. This is shown by the G.I. series. Here is a huge abscess cavity which has drained spontaneously into the fourth portion of the duodenum. This was an alcoholic patient who developed acute pancreatitis and then had a long septic course. In the course of work-up and repeated G.I. series, this abscess cavity was discovered. Very shortly after this x-ray was taken, all the symptoms subsided, and the patient went on to make an uneventful recovery.

I also had the opportunity in 1950 to study a patient of Dr. Leon Starr and Dr. Arthur Touroff. This patient was a very successful business executive who also was an alcoholic and who developed a fulminating acute pancreatitis. He was explored with the mistaken diagnosis of perforated ulcer and found to have a necrotic pancreas. During the exploration most of the pancreas sloughed out. Being intrepid and perhaps far in advance of their time, the surgeons went ahead and resected as much of the necrotic pancreas as possible. The patient was left with only the tail of the pancreas and a bare duodenum, and a visible common duct.

This patient went on to make an uneventful recovery. I saw him approximately six months postoperatively. He had an external pancreatic fistula that was draining 7 to 14 ounces a day. I was asked to study him because of my interest in pancreatic secretion. I performed a secretin test through the fistula. He secreted 39 cc from the fistula with 84 milliequivalents per liter, and the enzyme content was 4.3 units per kilogram. It then occurred to me that I ought to repeat the study with a tube in the duodenum. I did so and from the duodenum collected a much smaller quantity of pancreatic juice with a bicarbonate concentration of 32 and a trace of enzymes.

I studied this patient over the course of several months and got less and less out of the fistula and more and more out of the duodenum. The bicarbonate concentration progressively rose to 101 milliequivalents per liter. Then one day, nothing emitted from the fistula and pancreatic juice was obtained from the duodenum only. It was assumed that this patient had somehow gotten his pancreatic juice back into the duodenum. Dr. Touroff did not choose to believe this. So we did a lipiodol injection of the fistula which outlined a direct tract from the tail of the pancreas into the duodenum. Shortly thereafter, the fistula closed spontaneously, and this man had neither steatorrhea nor pancreatic insufficiency nor diabetes. He went on to make a complete recovery. Thus, there is some tendency for pancreatic ferments to erode their way back into the gut which is very fortunate because nature may do a better job than we can do.

So much for the particular cases. I would like to take whatever time remains in reviewing our modern concepts and critically analyzing modern therapy for acute and chronic pancreatitis.



In acute pancreatitis, the immediate choice is a question of medical versus surgical therapy. I think now from a large series in the literature, that the almost universal opinion is that if the diagnosis of acute pancreatitis is made, medical therapy is preferable to surgical therapy. Why? The mortality of the medical series is far lower, ranging between 5 and 10 per cent and gradually edging toward 5 per cent as compared to the best mortalities in the surgical series, which range around 25 per cent. There is another important reason for the choice of medical therapy. Supposing you go in on a patient with acute pancreatitis, what are you going to do? This is a very important question, because we are not always able to make the diagnosis with accuracy and we find ourselves exploring a patient who does in fact have acute pancreatitis. What does one do at the time of operation? Here there is some diversity of opinion, but, again, I think the general tendency is to do nothing, because one cannot drain the pancreas. Doing a cholecystostomy is advocated by some in the hope of decompressing the biliary tract and in this way somehow ameliorating the biliary factor in pancreatitis. But if the patient has an alcoholic history, I think that doing a cholecystostomy complicates the picture because then you are going to end up having to do a cholecystectomy which might not be required in the first place. I think that most surgeons, upon making a diagnosis of acute pancreatitis, do not operate.

What does the medical therapy consist of? It consists of a number of unrelated symptomatic therapies. First of these is to treat pain. I think the treatment of pain has been much overemphasized in the literature. We have great controversies as to whether or not morphine should be given because morphine, at the very instance of pain relief, will exacerbate or compound the mechanistic basis for the development of pancreatitis. Spasm of the duodenum and sphincter therefore increases pancreatic duct pressure which we are trying to decrease.

Some have advocated Banthine or Probanthine in the hope of suppressing pancreatic secretion and in this way decreasing pancreatic duct pressure. There have been various other suggestions such as procaine intravenously, nerve blocks, et cetera.

I have never used any of these. I think the best relief for pain can be obtained by emptying the stomach by putting the patient in nasogastric suction. A good deal of the pain is not necessarily related to the pancreas but to the ileus from which the patient is suffering. I do not remember having ordered morphine or Demerol or any analgesic in years.

The second problem that immediately confronts the therapist is the treatment of shock, because this is an outstanding problem and it is associated with fluid, electrolyte and blood volume deficit. The recognition of these deficits have been responsible for the decreasing mortality observed in the acutely ill patient. We all now realize that the blood volume deficit may be in the range of 33 per cent of the blood volume. Thus, one transfusion may not be nearly enough. These patients require several transfusions of blood, plasma, or any plasma expander because they have a distinct and large blood volume deficit.

The treatment of electrolyte and fluid replacement depends upon the indi-

vidual patient. When you see the patient he is excessively dehydrated. A great deal has been written about the problem of hypocalcemia and the development of tetany. These patients might require intravenous calcium gluconate. Indeed, a depression of the blood calcium has been taken by many to be a poor prognostic sign. I think that this concept is correct. In some instances, a calcium gluconate itself will not suffice to relieve the hypocalcemic tetany and in these patients Parathormone has been observed to be beneficial. The explanation is that the ultimate effect, the electrolytic imbalance, depends not so much on the total calcium but on the ionic calcium and Parathormone is capable of elevating ionic calcium whereas calcium gluconate might not be.

The third medical approach is directed toward those factors which will depress pancreatic secretion in the hope that the decreased secretion of ferments into the peripancreatic tissue would decrease the essential pathology and prevent progression of the disease. I think one of the most important factors in this respect is still intubation because this will decrease the gastric acidity and, therefore, prevents continued formation of endogenous secretin. The patient must not be fed, because if we want to suppress pancreatic secretion, the duodenum must be kept relatively alkaline in order to suppress endogenous secretin formation.

We have studied Diamox as a pancreatic secretory depressant. While it is true that this will suppress and block pancreatic secretion by making it impossible for the pancreas to synthesize bicarbonate and therefore eliminating the fluid component, it is a matter of debate as to whether it is useful in a patient with acute pancreatitis. We have not used it extensively in patients with electrolytic imbalance. However, Diamox has been useful in clearing up pancreatic fistulae by decreasing fluid output.

A number of anticholinergics have been used. Atropine is probably contraindicated and Banthine and Probanthine are of little value because in the concentrations used they do significantly affect pancreatic secretion. The corollary argument is that they do affect gastric acid and in this way may act as an indirect suppressant to pancreatic secretion, but to impose atropinism on a patient who is dehydrated and has an electrolytic imbalance for this relatively minor effect, which can be achieved with intubation, is unnecessary.

Radiotherapy has been used by some in the hope that it will produce an immediate suppression of pancreatic secretion, but I can quote no large series on its value.

Although we have spoken about intubation as a means of suppressing pancreatic secretion, I think it should be given a grouping of its own because the therapy of ileus is very important in acute pancreatitis. These patients have paralytic ileus which must be relieved and intubation is very useful in this respect, as well as in the relief of pain and distention.

There are two other facets which must be considered in the treatment of acute pancreatitis. First, the use of antibiotics is extremely important because this helps prevent the development of abscess formation. I must say this abscess is one of the first I have seen in a long time. Pancreatic abscesses were much more

common before the days of antibiotics. The pathology of pancreatic necrosis is, of course, sterile. But the tendency is for areas of liquefaction of blood and tissue to become secondarily infected so that a good percentage of the acute hemorrhagic necroses go on to abscess formation and then the usual course involves incision and drainage repeatedly as more and more of the pancreas is sloughed. Finally, if one does achieve sterilization of the abscess, one is generally left with a pancreatic fistula which then has to be treated. Unlike the presented case, not all of the patients produce a spontaneous fistulization to the gut.

I do not think we could leave the subject of acute pancreatitis without some mention of the antienzymes, because Trasylol is extremely popular at the present time. It is not an original concept. The introduction of albumen was originally for its antitrypsin effect. It was only when its blood volume expanding effect was appreciated that we did go on to other blood expanders. In addition, soy bean trypsin inhibitors and some antilipase inhibitors have been used. Most of these, with the exception of Trasylol, have been too toxic. Trasylol, an antitrypsin inhibitor, which does not affect pancreatic secretion, apparently does affect the activity of enzymes in the blood and tissues. I think only time and further experience will tell whether this drug is going to be of use in the treatment of acute hemorrhagic pancreatitis. However, I am not overenthusiastic, because I have seen nothing experimentally or clinically that has convinced me that it is worth while.

If the therapy of acute pancreatitis leaves much to be desired, that of chronic pancreatitis is in a far more unsatisfactory state.

*Question:* Could we have a word about the complications of acute pancreatitis at this point? What do you do about an abscess?

*Dr. Dreiling:* Incision and drainage.

*Question:* If there is an external fistula found in such an abscess and drainage?

*Dr. Dreiling:* If we have an external fistula, I think, at the present time, one should treat it with Diamox and wait. These fistulae usually close spontaneously. They were much more troublesome in the past. There is a series of operations of internal diversion of fistula into the stomach, into the duodenum, or by Roux-en-Y into the jejunum. I do not think this is necessary at the present time and I have not treated a fistula surgically in about ten years.

In chronic pancreatitis what can we do? The first factor that we have to consider is diet. If this pancreatitis is related to alcoholism, I maintain very firmly that if you cannot cure the alcoholism, you cannot cure the patient. I would not subject an alcoholic patient who refuses to give up drinking to a series of surgical procedures because I do not think that this would benefit him. Of course, we place all patients with chronic pancreatitis on relatively bland diets, but the diet itself is of little importance unless there is evidence of external pancreatic secretory defect in the form of steatorrhea, in which case a low fat diet is of considerable help. These patients may be able to handle small amounts of fat whereas a normal fat diet or a high fat diet would lead to steatorrhea.

Whether or not pancreatin is necessary depends, of course, upon how severe the pancreatic enzyme defect is and what the diet administered has been. In

most instances you can, by using a low fat diet, obviate the necessity for supplementary pancreatin. But if you cannot, and if pancreatin is necessary, it must be given continuously around the clock. Grossman has very clearly demonstrated that this, in large doses, is the most effective way. Available preparations are pancreatin, Viokase, and papaya extract. Why papaya extract, which does not contain a lipase should be effective, I cannot answer. Indeed, in patients who have steatorrhea, it is the lipase deficiency which is salient. These three preparations seem to be equally effective. How frequently do I use replacement therapy? I prescribe pancreatin perhaps once a year. I think diet itself is probably the most effective therapy for steatorrhea.

If there is biliary tract disease, the best results are obtained in these patients with eradication of the biliary tract disease. This means not only taking out the gallbladder but also exploring the common duct. If there are common duct stones, one must remove them. If there is a stenosis of the sphincter of Oddi, I think this must be treated by sphincterotomy. If there is no stenosis, sphincterotomy is not indicated. Finally, if there is diffuse calcification of the gland, an attempt at sphincterotomy would be inadvisable because there must be multiple areas of stricture within the duct system. Furthermore, there are some patients who develop a longitudinal stricture of the distal common duct. Here a sphincterotomy is useless because it cannot relieve a longitudinal area of obstruction. From bitter experience, we have come to the conclusion that a biliary diversionary procedure such as a choledochoduodenostomy or a jejunostomy is advisable in order to protect the patient with secondary common duct stricture from the development of biliary cirrhosis.

Pain is an outstanding symptom of chronic relapsing pancreatitis. Drug therapy leaves much to be desired. Many of these patients unfortunately become morphine addicts. We have tried Teandaryl, which is a butazolidin derivative, and Piptal, an anticholinergic, with some relief of pain.

Nerve surgery, such as sympathectomy and vagisection, has also been advocated. The immediate results with sympathectomy were good, that is, pain was relieved for a period of one or two years, during which time the patients might have exacerbations of their chronic pancreatitis but not with pain. Recurrences under such circumstances would be shown up by elevations of the blood amylase, with a fever, and with ileus, but without pain. Almost invariably, either due to regeneration of the sympathetics or some other mechanism, pain has recurred. I think this form of treatment is now used less frequently.

Vagisection, itself, as a sole treatment has not been used, but has been utilized in conjunction with a diversionary procedure such as gastroenterostomy. This is done in the hope of diminishing the pancreatic response to an ordinary meal, on the basis that the pain is due to ductular dilatation in a gland secreting against obstruction. I do not believe that the results have been sufficiently good to warrant this form of therapy alone.

Some have advocated pancreatic duct surgery in the relief of pancreatic pain. The following slides illustrate some of the complicated procedures that have been advised. This slide illustrates surgery for pancreatic cysts. It shows a cyst

in relationship to the posterior wall of the stomach. If a cyst is so located, why should one go to the trouble of doing a cystoenterostomy Roux-en-Y? The cyst is plastered up against the stomach. You probably would have to dissect this septum, a technically difficult procedure which is not necessary at all. A simple cystogastrostomy will give you adequate drainage with complete relief. The cyst will rapidly diminish in size so that after several weeks you may not be able, by G.I. series, to show a cavity. Usually at the end of three or four weeks you cannot even detect the previous cyst.

The next slide shows another pancreatic pseudocyst which here is not in relation to the stomach but presents in the greater peritoneal cavity. This type of cyst, of course, can be treated by a cystoenterostomy Roux-en-Y, and the next slide will illustrate such therapy.

The next slide shows a cystogastrostomy completed. It illustrates that the opening must be quite large, because it tends very quickly to become smaller. If you construct a smaller cystogastrostomy initially, secondary closure may require redrainage.

The next slide will show a cystoduodenostomy. After all, if the cyst is near the duodenum, why should we not take advantage of it and make the anastomosis between the cyst and the duodenum?

The next slide shows a cystojejunostomy without the Roux-en-Y principle. I think most of us, however, would do a Roux-en-Y.

I want now to discuss some of the techniques of attacking the obstructed pancreatic duct system itself. This is a procedure that goes back to about 1944. At Coffey's suggestion, the implantation of the pancreas with its open duct into the jejunum was performed in order to decompress the pancreatic duct system.

The next slide shows a suggestion made by Patrie, Pyle and Vale. Here the pancreatic duct was intubated and the prosthesis placed in an opening in the small intestine. These prostheses unfortunately tend to plug up so that they are not generally used.

Next is Cattell's approach. This goes back to the late forties and early fifties. You begin to see the development of the modern operation develop from this anastomosis between the mucosa of a dilated duct and the jejunum.

The next slide shows Bonta's original operation, proposed about four or five years before Duval's. One can see the formulation of the concept of retrograde drainage. If you cannot drain a multiply-obstructed pancreatic duct into the duodenum, one may drain it retrograde via a Roux-en-Y loop of jejunum. Most of the credit for the technique goes to Dr. Duval, who popularized this type of retrograde decompression. More and more as this operation has been done, there has been a tendency to resect more pancreas, so that the Duval operation must be considered a partial pancreatectomy as well as a retrograde drainage.

The more modern operations include Dr. Puestow's operation in which the fileting of the pancreatic duct with its anastomosis into a defunctionalized duodenum offers the hope that the multiple areas of obstruction which are producing the symptoms, the pathology, and the persistence of inflammation in chronic pancreatitis would be so drained. There have been a number of objections



to this procedure. One of the objections was raised by Thal, who said the pancreatic juice must not be diverted from the duodenum lest peptic ulcer develop. A number of these patients whose pancreatic juice has been diverted from the duodenum have, indeed, secondarily developed duodenal ulcer.

This is Dr. Gillespie's approach. The mobilized tail of the pancreas is anastomosed into the excluded antrum and a gastrojejunostomy is performed to the fundus of the stomach to restore gastrointestinal continuity. This procedure is a good way of developing gastric hypersecretion and ulcer because alkaline secretions are poured into an antrum. But it just gives you an impression of the complexity and diversity of approach which would again indicate to all of us that perhaps none of these methods is ideal.

Finally, in those patients whose symptomatology persists despite all surgical therapy, some surgeons have advocated a Whipple procedure or a total pancreatectomy. I admire Dr. Porter for his fortitude in subjecting a patient to a total pancreatectomy for a benign disease. Dr. Porter has reported a high rate of failure because these patients continue to have pain and return to alcoholism because of this unremitting pain.

Dr. Bower has suggested an operation which the French are now practicing, that is, subtotal resection of the pancreas, leaving a rim of pancreatic tissue so that the duodenum and the gastroduodenal arcades are not disturbed. Perhaps this will be the ultimate answer to the patient with chronic relapsing pancreatitis whose incapacity due to pain is unremitting and who has not responded to all of the lesser measures.

*Dr. Burrows:* Thank you, Dr. Dreiling, for a very complete discussion of this disease and its complications. I think we have time for a few questions.

*Question:* An important differential diagnosis exists between acute coronary and acute pancreatitis, because in one you are advocating fluid replacement with transfusion, and in the other we have the problem of not overloading. In some cases of pancreatitis have there been ECG changes?

*Dr. Dreiling:* Yes. I had a patient, a physician, who was sent to me by a medical man on the first day of an acute attack of pain which he was sure was pancreatitis. I was young enough at the time to perform a secretin test on him. He truly had a coronary and fortunately nothing happened. This is a real differential that will occur not infrequently. You cannot be certain from the electrocardiographic changes, because we have a number of patients with acute pancreatitis who come to post with ST segment and T wave changes in whom no myocardial infarction is found post mortem.

*Question:* Are patients with classical pancreatitis like the first patient, who showed decreased enzymes during the secretin test, truly capable of having an attack of acute pancreatitis, as we know, with elevated blood enzymes?

*Dr. Dreiling:* Yes, even patients with burnt-out pancreatitis can have recurrent attacks with blood enzyme elevations.

Although such patients are putting out small quantities of enzymes into the duodenum, this does not mean that all acini in the gland are destroyed because the pathology is spotty. What you see in these patients is the end result of



multiple recurrent cycles of focal necrosis followed by attempts at regeneration, fibrosis and calcification. Thus, even in a gland with advanced chronic pancreatitis there are areas of normal acinar tissue surrounded by fibrosis—these patients can produce enzymes and can suffer real acute pancreatitis.

*Question:* In your discussion you mentioned Trasylol. In Boston, Dr. George Nardi told me that they treated fifty or sixty patients with Trasylol and they are very satisfied with the results. In my country, our medical faculty has treated almost 100 patients with acute pancreatitis. Trasylol is very effective in acute pancreatitis and also in recurrent pancreatitis. Trasylol is useful in decreasing pain and in aborting attacks.

*Dr. Dreiling:* I did not say that Trasylol was useless. What are your controls to say that Trasylol, that is being used in addition to other modalities, is producing a specific alteration in the course of an acute pancreatitis or even in the course of chronic pancreatitis?

*Question:* To tell the truth, sir, we did not compare, simultaneously, patients treated with Trasylol and patients treated without Trasylol. But, if we compare our results from the time when we treated our patients with Trasylol with our results before Trasylol treatment, the results with Trasylol are much better.

*Dr. Dreiling:* This is a clinical impression. I am sorry, Doctor, I cannot accept a clinical impression as proof. I think you have to have more evidence. It is difficult to come to a conclusion in therapy of this disease. The clinical course is so variable that a patient can come in and die within twelve hours. A patient can come in and look like he is going to die and within twelve hours walk out of the hospital. Even if one is capable of forming a clinical impression of severity in such a protean disease with different etiologies, one cannot compare acute pancreatitis associated with biliary tract disease with the fulminating type that may occur with alcoholism. As long as we are dealing with clinical impression, I am willing to say go ahead and try Trasylol. When we have ten years of experience we may know the answer. We have all lived through a period in which propylthiouracil was hailed with equal enthusiasm, but I do not know of any clinic that uses propylthiouracil today.

*Question:* Is there any side effect from the use of that drug?

*Dr. Dreiling:* None of which I am aware. I do not think serious complication has been reported.

*Dr. Glisic:* We did not have any side effects during our treatment of our patients with Trasylol.

*Question:* What was the dosage you were giving?

*Dr. Glisic:* It is very important that you treat patients as soon as possible and with large doses. We give to our patients 30 or 40,000 units every day.

*Dr. Dreiling:* I think you have just struck on a terribly important and significant fact. I had the opportunity to work with Trasylol about three to four years ago. I can assure you that within the past four years the dosage recommended by the drug companies has increased tenfold. If such remarkable responses were obtained with a lower dose, why was it necessary to recommend increased dosage?

*Dr. Burrows:* I think this discussion could be left for future years actually to see how it truly evolves.

*Question:* I just wondered if you had mentioned the role of steroids in the treatment of acute pancreatitis?

*Dr. Dreiling:* I left that out. I am sorry. Here, again, is another debatable area. If you look at the literature of about three or four years ago, there was a great excitement that steroids would be a panacea and the salvation of patients with acute pancreatitis. I do not think there is any evidence now, clinically or experimentally, that would show that steroids alter the course of acute pancreatitis unless there is an adrenal insufficiency either pre-existing or unless the patient develops apoplexy of the adrenal. Otherwise, there seems to be evidence even to the contrary. Barr and others have reported acute pancreatitis and pancreatic necrosis in patients receiving steroid therapy. This has been observed in children. It has been seen at post mortem examination in patients on a steroid therapy for unrelated disease as in rheumatoid arthritis. We did a study in which the pancreatic secretion was altered by administration of steroids to the type of secretion that one sees in chronic pancreatitis. Because of this, I would be a little reluctant to routinely administer steroids to all patients with acute pancreatitis. But I would have no hesitation in a patient who looks as if he is in extremis, and I did so in one patient with survival.

*Question:* Before we leave this subject, I think it might be important to make you all aware, if you are not already, of the recent change in nomenclature that pancreatitis has undergone during the last few months.

There was a conference in Marseilles where everybody got together because it was getting to be quite confusing as to what we mean by different types of pancreatitis. Very briefly, they classified pancreatitis as acute or chronic and subdivided the acute phases into acute pancreatitis and acute relapsing pancreatitis. In the chronic cases the terms "chronically relapsing pancreatitis" and "chronic progressive pancreatitis" were suggested.

*Dr. Dreiling:* I would like to add for the record, I am not stimulated by this classification. I am appalled by it, because such classification goes back to clinical descriptive medicine. It is unscientific and I think we could better classify this disease complex by going back to the pathology and into the etiology instead, as the hepatologists did. This classification makes little subdivisions just as the hepatologists did with cirrhosis, so that all acute hepatitis was acute catarrhal jaundice and all chronic liver inflammation was cirrhosis. This is wrong. We first must learn to classify by etiology and pathology. Only then can we bring order out of chaos. Thank you very much.

(The meeting adjourned at twelve-ten P.M. o'clock.)

# Endemic Shigellosis in the Underprivileged Community Served by Greenpoint Hospital

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Shigellosis, which manifests itself by fever, abdominal pain, tenesmus, and frequent watery stools that may contain mucus, blood or pus, results from infection by a member of the genus *Shigella*. Unlike the severe type of infection caused by *Shigella dysenteriae*, which is attended by a high mortality rate in India and Japan and is fortunately very rare in the United States, the disease observed in this country involving *Shigella sonnei*, *Shigella flexneri* and *Shigella alkalescens-dispar* is relatively mild and localized almost entirely to the gastrointestinal tract. Some question exists with regard to the taxonomic status and pathogenicity of the latter species. Although included within the genus *Shigella* in Bergey's *Manual of Determinative Bacteriology*, some authoritative sources consider it a member of the genus *Escherichia*. Considering the possible potential pathogenicity of *Shigella alkalescens-dispar*, all of the strains included in this report were isolated from patients suffering from persistent diarrhea, some with fever, others without, because of which symptoms a stool culture was requested. Its pathogenic capability is further attested to in the literature (1, 2).

Shigellosis is spread via the oral route. Such common food products as poultry, fish, eggs, etc., as is the case with salmonellosis, are usually not primarily involved in its transmission since the disease is limited to man almost exclusively and does not involve lower animal species. These products, however, may be secondarily implicated by virtue of contamination by a patient or carrier. The epidemic form, as seen in orphanages, institutions or military installations, is often traced to some common food source contaminated by a carrier in the food preparation center, which then results in widespread dissemination through the institution involving many persons. The endemic form, by comparison, is limited to sporadic cases among individuals or members of a family. Low standards of environmental sanitation and personal hygiene, which are more apt to prevail among lower socio-economic groups, play a significant role in the transmission of this disease.

The exact incidence of shigellosis is unknown. Diarrhea, its prime symptom, is a common, usually self-limited occurrence, the precise etiology of which can only be established by means of stool culture. The number of requests for this laboratory procedure is dependent in the main upon the suspicion of the examining physician and the availability of laboratory facilities. In actual practice, requests for stool culture are usually apt to be ordered only in the presence of an outbreak or when the symptoms fail to respond to therapy and persist. Furthermore, sporadic cases as compared to outbreaks (3, 4, 5) are rarely reported in the

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literature so that the actual incidence of endemic shigellosis is particularly difficult to ascertain.

One hundred and fourteen positive isolates of *Shigella* were obtained at the Greenpoint Hospital in Brooklyn, N. Y., in a one-year period extending from April 1, 1963, to March 31, 1964, thereby indicating a considerable prevalence of shigellosis in the community. Identification of each strain included in this report was verified by the New York Salmonella Center at Beth Israel Hospital, New York, N. Y. As a basis for comparison, only 106 cases of bacillary dysentery were reported to the New York City Health Department for the entire city of New York in 1962. This high incidence, however, does not necessarily represent a sudden marked increase for this period but may merely reflect an endemic situation brought to light. A consideration of a number of pertinent factors forms the basis for the present report.

Greenpoint Hospital is a small, acute, municipal hospital located in an area containing many industrial establishments as well as low income residences. Its bed capacity is only 150, but its out-patients' department, particularly its pediatric clinic, is extremely large and active and is the main source for the medical care of the surrounding community. The population from which its patients are drawn consists of mainly Negroes or Puerto Ricans in approximately equal numbers, with a small proportion of patients of Polish, Italian, Russian, Czech, German and Austrian origin. The vast majority of patients are in the relatively low income group.

The age, sex and ethnic origin of the 114 infected patients are shown in Table I. As may be observed from the table, most of the patients were children, 79% being under 12 years and 60.6% under 5 years of age. However, a greater degree of infection may be prevalent among adults than is indicated by these data. Adults do not seek medical care for themselves as readily as they do for their children, especially since the disease is mild and self-limited in nature. An indication of adult prevalence is provided by the finding of four positive isolates in the course of a limited survey among 50 pregnant mothers attending the prenatal clinic, an approximate incidence of 8%. Furthermore, 9 cases occurred in auxiliary nursing personnel, all of whom live in the neighborhood, although the possibility that their infection might have been acquired in the course of caring for patients cannot be excluded. Three other adult isolates were parents of children with positive cultures, and may have been the source of their children's infections. All of the above provides evidence for a considerable prevalence of infection among adults as well as children. As for ethnic origin, patients of Puerto Rican origin predominated (60.5%), followed by Negroes (32.5%), whereas the remaining group furnished only 7.0% of the total.

Additional evidence for the endemicity of this disease in the community is provided by the fact that 89 of the 114 cultures were obtained from out-patients. Of the 25 isolates from hospital patients and staff, 9 positive cultures, as mentioned previously, were obtained from auxiliary nursing personnel living in the community. Of the remaining 16 strains recovered from hospital patients, 10 were in recent newborns who could very well have been infected by their mothers,

who in turn might have been already infected prior to their admission to the hospital for delivery.

The number of positive isolations obtained each month is depicted in Figure 1. As may be observed from the graph, cases continued to appear throughout the entire survey period and this scattered distribution is further evidence for the existence of community infection. The number of isolates per month varied, the largest number occurring in the post-summer period from September through December inclusive. An additional flare-up was noted in March 1964.

A small epidemic accounted for a total of 8 cases, all infected with *Shigella flexneri*, Group 6. It started with a newborn child who infected three other new-

TABLE I  
*Age, Sex and Ethnic Origin of 114 Patients in Whom Shigella Strains Were Isolated*

	Total (114)	Per cent total	Shigella species		
			Sonnei (45)	Flexneri (27)	Alkalescens- dispar (42)
Age					
Newborn	10	8.8	1	4	5
Under 1 yr.	23	20.2	9	8	6
1-5 yrs.	36	31.6	14	5	17
6-12 yrs.	21	18.4	14	3	4
13-20 yrs.	7	6.1	1	1	5
21 yrs and over	17	14.9	6	6	5
Sex					
Male	57	50.0	27	10	20
Female	57	50.0	18	17	22
Ethnic origin					
Negro	37	32.5	22	6	9
Puerto Rican	69	60.5	19	20	30
Other	8	7.0	4	1	3

borns as well as three nurse's aides working in the newborn nursery within a short period before the exact nature of the first child's illness could be ascertained. The mother of the original newborn may very well have been the primary source for the entire outbreak since she was found to harbor the same *Shigella* species as her child.

A number of medical and technical factors contributed toward this apparent increased incidence over that of previous years. The Mount Sinai Hospital assumed medical responsibility for Greenpoint Hospital in July of 1962, as a result of which affiliation markedly expanded medical and laboratory facilities became available to the latter institution after a breaking-in period of several months. The added number of attending physicians participating in patient care resulting from this responsibility in turn resulted in many more stool cultures being requested in suspicious cases and these requests could now be readily accom-

modated by the laboratory. A technical laboratory procedure was also newly introduced which had not been employed previously that also contributed toward the relatively large number of positive identifications. This consisted of boiling all suspicious cultures for 30 minutes prior to their being slide-tested for agglutination with a *Shigella* antiserum. Although a suspicious *Shigella* organism

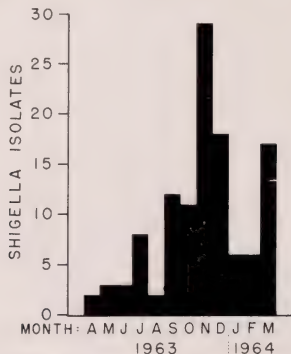


FIG. 1. Monthly distribution of 114 *Shigella* isolates.

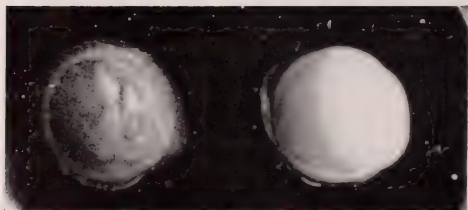


FIG. 2. (Left) Positive slide agglutination with *Shigella flexneri* antiserum of *Shigella flexneri* strain after boiling for 30 minutes. (Right) Negative agglutination with same culture unboiled.

may be tentatively identified on the basis of biological, morphological and biochemical properties, its exact nature can only be definitively established by its positive agglutinability with specific *Shigella* antiserum. Some strains, however, are not agglutinated or agglutinate poorly with specific antiserum unless first heated to 100°C for 15 to 30 minutes because of the presence of a heat labile inhibitory substance in the culture (16). A comparison of the results of agglutination tests performed with and without preliminary boiling of the culture revealed that whereas all 45 *Shigella sonnei* strains agglutinated with specific antiserum



irregardless of whether or not they had been boiled, only 7 of the 27 *Shigella flexneri* strains and only 8 of the 42 *Shigella alkalescens-dispar* strains were agglutinated by their specific antisera without boiling. Thus, had not this technical procedure been initiated, 54 of the 114 strains in this series would have been considered as some organism other than a *Shigella* and missed. A negative agglutination reaction observed with an unboiled *Shigella flexneri* culture as compared to the positive serological reaction obtained with the same strain after boiling is illustrated in Figure 2.

It is of interest that during the same period under consideration, only 36 positive cultures for *Salmonella* were obtained as compared to the 114 *Shigella* isolates. Of the former, 27 were obtained from out-patients and 9 from hospital patients, thereby indicating the existence of a salmonellosis reservoir in the community as well, although to a lesser degree. Of 36 *Salmonella* isolates, 18 were *S. typhimurium*; 6, *S. heidelberg*; 3, *S. enteritidis*; 2 each of *S. newport* and *S. typhi*, and one each of *S. saintpaul*, *S. infantis*, *S. blockley*, *S. kentucky* and *S. oranienburg*.

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# Salmonella Derby Infections After Gastrointestinal Surgery

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Salmonella infections as a cause of postoperative diarrhea, occurring within a short time after surgery, have rarely been reported in the past. The recent epidemic of hospital-associated infections with *Salmonella derby* (1, 2), which began early in 1963, should alert the surgeon, gastroenterologist and internist to this possible postoperative complication.

The most common causes of diarrhea occurring shortly after surgery are usually felt to include staphylococcal or pseudomembranous enterocolitis, post-vagotomy syndrome with hypermotility and "dumping," a surgically created blind loop or fistula, monilial and viral infections, reactions associated with antibiotics and a "non-specific" variety (3).

The purpose of this communication is to outline the clinical picture of salmonellosis occurring in the immediate postoperative period. Thirteen such occurrences were noted from March 1963 to February 1964 at The Mount Sinai Hospital in New York City. Three illustrative cases follow. Table I demonstrates the features in these thirteen cases.

*Case 1.* The patient was a 52 year old man with a fifteen-year history of duodenal ulcer disease. Because of uncontrollable and persistent abdominal pain, he underwent surgery on March 8, 1963. A subtotal gastrectomy with vagotomy and Billroth II anastomosis was performed.

The postoperative period was uneventful until the seventh day when the patient developed malaise and temperature to 104°F accompanied by five loose, nonbloody bowel movements. The white cell count was 13,600 and the patient became quite ill. A stool culture was taken at the onset of the diarrhea and later reported to contain *S. derby*. Blood and urine cultures were negative.

The illness was unresponsive to intravenous fluids and deodorized tincture of opium. On the third day of fever and diarrhea, chloramphenicol and methicillin were begun as a staphylococcal enteritis was suspected. The methicillin was discontinued upon receipt of the culture report. The diarrhea continued for a total of seven days. At the time of discharge, three weeks after surgery, the patient was asymptomatic but *S. derby* continued to be present in cultures of the stool.

*Case 2.* A 22 year old woman with regional ileitis involving the terminal ileum was admitted on December 23, 1963, with a one-year history of diarrhea, weight loss and abdominal pain. She was treated with prednisone, ACTH, neomycin and sulfathalidine with little response. An ileocolic resection was performed on January 15, 1964. Postoperatively penicillin, streptomycin, chloramphenicol and hydrocortisone were given. During the first postoperative day a grand mal

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TABLE I  
13 Cases of Postoperative Salmonellosis

Case no.	Operation	Date 1st pos. culture	Postop. day of illness	Max. temp. F.	Diarrhea	WBC cu./mm	Culture site	Duration symptoms (days)	Stool culture at discharge
1	Billroth II and vagotomy; duodenal ulcer	3-19-63	7	104	Yes	13,600	Stool	7	Pos.
2	Appendectomy	3-20-63	10	99	Yes	16,900	Stool	2	Neg.
3	Billroth II and vagotomy; duodenal ulcer	3-27-63	6	102	Yes	8,600	Stool	18	Neg.
4	Ileocolic resection; carcinoma	3-29-63	8	105	Yes	14,300	Stool	3	Pos.
5	Pyloroplasty, local resection; benign gastric ulcer	4-3-63	7	101	Yes	6,200	Stool	2	Pos.
6	Billroth II; carcinoma of stomach	9-27-63	8	103	Yes	13,800	Stool	3	Pos.
7	Billroth II; carcinoma of stomach	1-10-64	9	102	Yes	Not done	Stool	4	Not done
8	Ileocolic resection; ileitis	1-20-64	8	105	Yes	19,600	Stool	3	Not done
9	Splenectomy; Hodgkin's	1-27-64	7	103	Yes	13,000	Urine Stool	Expired 17th postop. day	Expired
10	Cholecystectomy; Billroth II; gastric ulcer	1-27-64	12	101	Yes	14,000	Stool	3	Not done
11	Nephrostomy; prostatic carcinoma	2-19-64	7	101	No	9,200	Stool Urine	0	Pos.
12	Cholecystectomy	2-19-64	5	102	Yes	17,400	Stool	30	Pos.
13	Oophorectomy; cystadenoma	2-22-64	7	100	Yes	14,000	Bile Stool	3	Pos.

seizure was observed. This was felt to be related to the steroid therapy. On day 8, another seizure occurred and a temperature of 105°F was charted. This was accompanied by eight loose bowel movements during the next 36 hours. A leak at the anastomosis was feared. A white cell count was 19,600. Blood cultures were reported as negative but both stool and urine contained *S. derby*. The patient was treated with ten days of chloramphenicol after the positive culture was obtained. The urine culture may have been fecally contaminated. The patient was discharged with a negative stool and urine culture.

*Case 10.* This 58 year old man was admitted with symptomatic chronic cholelithiasis. He had a cholecystectomy on January 6, 1964. At the time of surgery, a benign gastric ulcer was noted and a subtotal gastrectomy with a Billroth II anastomosis was performed. There was no vagotomy.

Twelve days after the operation anorexia, nausea, malaise and diarrhea developed. There was a temperature of 101°F and four loose stools. Salmonellosis was suspected by the attending physicians. A gram stain of the stool revealed no gram-positive cocci. White cell count was 14,000. A stool specimen was sent for culture and *S. derby* grew out. There were negative urine and blood cultures. The patient was treated with chloramphenicol for one week and discharged asymptomatic. A follow-up stool culture was not obtained.

#### DISCUSSION

In a review of the literature similar immediate postoperative salmonellosis has been reported in only eighteen previous instances. The organisms involved were *S. enteritidis* (4), *S. oranienburg* (5, 6), *S. montevideo* (5), *S. typhimurium* (5), *S. suipestifer* (7), *S. typhosa* (8), *S. muenchen* (9) and *S. choleraesuis* (10). The majority of these older cases involved surgery of the gastrointestinal tract. Ten of the thirteen cases reported in the present series involved gastrointestinal procedures.

The characteristics of postoperative salmonellosis with the *S. derby* organism are recorded in Table I and in the three case reports. The Table also reveals the series to be divided into two groups by date of infection. Case 6 occurred in September, whereas the other twelve cases occurred either in the March–April 1963 period or at the end of the twelve-month period. In many instances toward the close of the year, the diagnosis was entertained before the culture was reported.

As noted in Table I, gastrointestinal surgery was performed in ten of the thirteen instances. Oophorectomy, nephrostomy and splenectomy were the exceptions. Although infections became manifest from the fifth to the twelfth postoperative day, the majority of cases appeared on the seventh and eighth day. There was usually fever accompanying the diarrhea (Table I). The diarrhea was nonbloody and occurred from two to eight times in a twenty-four hour period. Only Case 11 was free of diarrhea.

A leukocytosis of 13,000 to 20,000 was present in nine of the patients. The organism was recovered in the stool in twelve instances. The urine was positive

for *S. derby* twice and the bile was positive in the one case in which it was cultured. All blood cultures were sterile.

The duration of the symptomatic illness was from two to four days in most patients but the severity of the illness was mild. Most of the cases were better by the time definitive therapy was instituted; however, Cases 3 and 12 had protracted illnesses. Death occurred once, in a moribund patient, post-splenectomy for Hodgkin's disease. This death rate is in marked contrast to the older cases of postoperative salmonellosis caused by other species in which one-third of the patients succumbed. As of June 1963, the Public Health Service reported 16 deaths in association with the northeastern United States outbreak of *S. derby*. One of these cases was a 53 year old man who had "G.I. surgery" in Pennsylvania.

Table I also shows that seven cases continued to harbor *S. derby* in the stool despite vigorous and prolonged antibiotic therapy. The development of a chronic carrier state constitutes both a public health hazard and a danger for the individual. Previous workers have noted that in 182 cases of *S. derby* 22% of the individuals became chronic carriers (11). Chloramphenicol along with other antibiotics in some instances was used in each of the postoperative cases in this series. A good clinical response was obtained each time but neomycin in addition was utilized twice to eradicate the organism.

The well-recognized predisposition of a patient with a gastrectomy to *Salmonella* (12) is reflected in this series, since six of the group had gastric surgery. Whether the same predisposition is in effect in short-term postoperative states as in patients infected months to years after surgery is not clear.

It has been suggested by Waddell *et al.* (12) that the changes in gastrointestinal status that might predispose these patients to infection include achlorhydria and rapid gastric emptying. General debility and altered bacterial flora postoperatively may also play a role. Gastrointestinal disease without surgery apparently does not predispose to *S. derby* infections, since in addition to the thirteen cases reported here, there were eleven other cases at this hospital associated neither with surgery nor gastrointestinal disease.

In a discussion of predisposition, the use of antibiotics and steroids are to be considered. *Salmonella* infections may be facilitated by antibiotic alterations of the intestinal bacterial flora (13, 14). When a patient undergoes intestinal surgery, and may or may not have been on antibiotics, Bennett and Hooke (13) state that "non-specific diarrhea" or staphylococcal enteritis should not be diagnosed until proper bacteriologic investigations are completed. Similarly, it has been suggested that steroids depress resistance to *Salmonella* infections. In these thirteen cases, antibiotics were used seven times, steroids three, and in three patients both antibiotics and steroids were used prior to, or immediately after surgery.

The high incidence of *S. derby* occurring during 1963 has brought this organism to the attention of the physician, epidemiologist, bacteriologist and hospital administrator. According to the U.S. Public Health Service, almost 2,000 cases of infections with *S. derby* have been detected between March and December 1963 (2). Of these, the majority were hospital associated. Prior to this outbreak,

the relative incidence of *S. derby* represented 2% of all *Salmonella* infections reported. At the close of 1963, it accounted for 8 to 14% of all *Salmonella* infections.

The remarkable increase is obvious at this institution as well. For the ten-year period prior to March 1963, *S. derby* had been isolated but once (15); however, for the twelve months after that date, there were 24 cases. Thirteen of these 24 cases constitute the present experience with salmonellosis after surgery. During the same year, *Salmonella* infections with other species has occurred at the usual rate, and none have been in the postoperative period.

The epidemiological characteristics of *S. derby* outbreak in hospitals has been discussed by Sanders *et al.* (1). The outbreak began in March 1963 involving hospitals in the northeastern United States. A source of infection was later identified as raw or undercooked eggs to account for the sporadically appearing multiple cases in various parts of the hospitals. As a result, it was recommended that the eggs be withdrawn from the hospitals. The importance of *Salmonella* infections as related to animal food products, particularly eggs, has previously been emphasized (13, 14).

A secondary spread of the infection was noted in the end of June 1963 and the epidemiologic features of this wave suggested person-to-person spread of infection (12). Investigations at this institution parallel the experience as recorded by the Public Health Service.

#### CONCLUSION

Whether or not these cases represent new infections about the time of surgery or were flare-ups of carrier states in patients undergoing surgery can not be stated with certainty. The former possibility appears more likely, especially in the cases appearing in March and April 1963. The characteristic pattern of occurrence one week after gastrointestinal surgery in patients on the same services seems to point to a recently acquired infection.

Finally, it is concluded that in a patient undergoing gastrointestinal surgery who develops fever and diarrhea postoperatively, salmonellosis must be considered. The approach to the management of such a case is to perform a gram stain on the stool for staphylococcus. If the stain reveals predominantly gram-positive cocci, immediate antistaphylococcus therapy should be instituted (3) as a delay may prove fatal. On the other hand, if the smear does not indicate a staphylococcal etiology, then cultures should be obtained and antibiotic treatment withheld until the organism has been identified.

When *Salmonella* has been established as the causative agent, repeat stool cultures should be obtained to identify the chronic carrier state.

#### SUMMARY

Thirteen cases of *S. derby* complicating the immediate postoperative period are reported. The majority of cases involved surgery of the gastrointestinal tract. These infections were part of the widespread hospital-associated *S. derby* epidemic.



The patients became symptomatic one week after surgery with diarrhea, fever and leukocytosis. Most of the cases were mild and of short duration, but chronic carrier states may have been established in a number of instances.

An awareness of such postoperative infections is important for proper and early recognition and therapy.

#### ADDENDUM

Since completion of this one-year study four more cases of postoperative *S. derby* have been noted. Two were in gastrectomy patients, one occurred after a cholecystectomy, and one after an ileocolic resection.

#### ACKNOWLEDGMENT

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# **Studies on Biliary Flow and Composition in Man and Dog**

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Despite numerous studies the exact mode and site of formation of bile are as yet incompletely understood. On the basis of available data Brauer (1) divided the major biliary constituents into three classes according to the degree of concentration they experienced in their passage from blood to bile. According to this classification Class A includes the electrolytes as well as other substances appearing in bile in concentrations closely resembling those in plasma. It remained for Wheeler and Ramos (2) recently to advance the hypothesis that part of bile is constituted by an electrolyte fraction analogous to the electrolyte fraction of pancreatic fluid. This hypothesis was arrived at following the examination of bile specimen collected from unanesthetized cholecystectomized dogs following stimulation with secretin. Assuming endogenous secretin activity under physiological conditions their findings present evidence for an analogy between biliary and pancreatic secretion.

In view of the importance of this hypothesis, the present study was undertaken with the following objectives:

- A. Confirmation, if possible of Wheeler and Ramos' (2) results.
- B. Further investigation of biliary secretion and flow in an attempt to gather additional evidence for or against this hypothesis.
- C. Extension of these studies to humans.

## **MATERIALS AND METHODS**

Studies were conducted on seven human volunteers and five trained adult mongrel dogs. The human volunteers were all patients in whom a T tube with a balloon on the distal limb, capable of blocking the distal duet during bile collections, had been placed between one and two weeks previously during cholecystectomy and common duet exploration. All the dogs were prepared by cholecystectomy and installation of a Thomas duodenal fistula apparatus (3) between two and three months prior to study. In addition, one of the dogs was prepared by the placement of an Eek fistula by end-to-side portal vein-vena cava anastomosis.

Studies on the dogs were performed in the unanesthetized state with or without bile acid replacement. The bile acid used was a commercial preparation of Na Taurocholate by the Dajac Company. It was administered continuously intravenously as a 1% solution by the method described by Wheeler and Ramos (2) or intraduodenally as a 10% solution replacing excreted bile ml for ml. Bile

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was obtained by free flow through a glass cannula. Secretin studies were performed by examining the flow and composition of bile prior to and following the intravenous administration of 100 units secretin Vitrum obtained from the Karolinska Institute, Stockholm, through the courtesy of Professor E. Jorpes. Studies with Diamox were performed following the administration of 500 mg intravenously.

"Stop Flow" studies were conducted by raising the outflow tube connected to the glass cannula to sufficient height to permit spontaneous cessation of biliary flow. The height to which the bile column rose was usually between 12 and 18 cm above the papilla of Vater with the dog in the upright position. After periods varying between 5 and 12 minutes multiple specimens of 0.6–1.0 ml were obtained, their flow rate measured and their composition determined. These studies were also conducted following the administration of secretin and/or Diamox just prior to stoppage of the flow. The outflow tube contained approximately 0.4 ml which were mixed with the first specimen.

The human volunteers were given 1.0 units/kg of secretin Vitrum intravenously and specimens were collected prior to and for 20 minutes following the administration of the stimulant as the peak action occurred during this time.

Bile acids were determined by a modified Minibeck procedure according to the method of Singer and Litschen (4).  $\text{HCO}_3$  concentrations were determined by the method of Van Slyke and Neill (5) and chloride concentrations by the method of Cotlove *et al.* (6). Na and K concentrations were measured by flame photometry and osmolality with a Fiske osmometer. The formulas used by Wheeler and Ramos (2) to determine the volume of the hypothetical electrolyte fraction  $V_E$  and the concentrations of its Cl and  $\text{HCO}_3$  ions  $\text{Cl}_E$  and  $[\text{HCO}_3]_E$  will be referred to later and are as follows:

$$V_E = V \cdot \frac{[\text{Cl} + \text{HCO}_3]}{150} \quad (1)$$

$$[\text{Cl}]_E = \frac{V}{V_E} \cdot [\text{Cl}] \quad (2)$$

$$[\text{HCO}_3]_E = \frac{V}{V_E} \cdot [\text{HCO}_3] \quad (3)$$

## RESULTS

### 1. General Considerations

A. The dependency of biliary flow on the enterohepatic circulation of bile acids has previously been noted (7). Resulting difficulties in obtaining adequate specimens for examination prompted the adoption of the technique of continuous intravenous Taurocholate infusion by Wheeler and Ramos (2). In our experiments the progressive depression of biliary flow in otherwise healthy dogs was not as profound and rapid and permitted experiments of one and a half to two hours duration with reasonable accuracy (Table I).

B. Measurement of concentrations of bile acids. Na, K, Cl and  $\text{HCO}_3$  of 65 bile specimens confirmed the relationship found by Wheeler and Ramos (2) which consists of Taurocholate concentration =  $([\text{Na}] + [\text{K}]) - ([\text{Cl}] + [\text{HCO}_3])$  (Table II).

C. Measurement of the osmolality of 25 specimens demonstrated the isosmolality of bile with plasma despite marked variations of the concentrations of the separate ionic constituents (Tables I and II).

D. Instead of intravenous replacement of bile acids according to the method of Wheeler and Ramos (2) intraduodenal replacement with a 10% solution of Na Taurocholate ml for ml was utilized in approximately half the experiments. This method was found to be as effective in the formation of a steady state as the intravenous one.

TABLE I

*The Effect of Secretin on Biliary Flow and Composition*  
(Representative Dog Experiment No. 2061-1)

Number of consecutive specimen	Time in min	Volume in ml	$V_E$	Cl in mEq. L	$\text{HCO}_3$ in mEq. L	$\text{Cl}_E$	$[\text{HCO}_3]_E$	$\text{Cl}_E + [\text{HCO}_3]_E$
1	5	8	3	44.9	10.8	121.3	29.2	150.5
2	30	2.2	0.8	40.6	15.6	113.6	42.1	155.7
<i>Secretin Vitrum 100 units intravenously</i>								
3	15	4.8	3.4	62.9	46.5	88.1	75.1	163.2
4	15	3.0	2.9	72.9	75.9	73.7	76.7	150.4
5	15	1.8	1.1	55.8	36.3	89.3	58.1	147.4

It is seen that despite the absence of bile acid replacement a marked effect of secretin was present. In this case rising  $\text{HCO}_3$  and falling Cl concentrations coincide with increased flow rates.

$V_E$ ,  $\text{Cl}_E$ , and  $[\text{HCO}_3]_E$ —calculated hypothetical electrolyte fractions according to formulae of Wheeler and Ramos (2).

## 2. Common Duct Bile

The presence of 5–10 ml of bile in the common duct at the beginning of the experiments as noted by Wheeler and Ramos (2) was confirmed. The composition of this bile is depicted in Table II. We too noted high Taurocholate, Na and K concentrations but were particularly impressed by the low values of Cl and  $\text{HCO}_3$  concentrations. When the Cl and  $\text{HCO}_3$  concentrations of the hypothetical electrolyte fraction are calculated according to the method of Wheeler and Ramos (2) a tendency to approach plasma levels is noted.

## 3. The Effect of Intravenous Secretin

The effect of intravenous secretin on biliary flow and composition was tested in eleven dog experiments and with seven human volunteers. In all cases the

previously described (8, 9) increases of flow rates with decreases of bile acid concentrations were noted. This effect was equally present in acute experiments without bile acid replacement (Table I).

We too were able to demonstrate marked elevations of Cl and  $\text{HCO}_3$  concentrations for short periods following administration of secretin. It was noted however that even when the hypothetical electrolyte fraction was calculated according to Wheeler and Ramos (2) its volume increases and its  $\text{HCO}_3$  concentrations were far below those observed in pancreatic fluid following administration of similar amounts of secretin. The tendency of the Cl and  $\text{HCO}_3$  concentrations of this fraction to equalize at approximately 75 mEq/L, as depicted graphically by Wheeler and Ramos (2), was noted by us as well (Tables I and II).

TABLE II  
*The Composition of Common Duct Bile*

Dog experiment no.	Na mEq/L	K mEq/L	Calculated Taurocholate mEq/L	Cl mEq/L	$\text{HCO}_3$ mEq/L	Cl <sub>E</sub>	$[\text{HCO}_3]_E$	$\text{Cl}_E +$ $[\text{HCO}_3]_E$
2060-1	206	7.4	153.3	48.1	12	120.2	30	150.2
2060-2	244	8.4	212	32.5	6.9	120.25	25.5	145.8
2060-7	192	7.2	143.5	44.9	10.8	121.3	29.2	150.5
2061-1	192	7.2	143	40.6	15.6	113.6	42.1	145.7
2061-2	200	7.4	162.5	32.3	12.6	100.1	39	139
2061-3	230	9.1	195.1	27.8	16.2	94.3	55	149.3

It is readily seen that concentrations of Cl and  $\text{HCO}_3$  of the hypothetical electrolyte fraction tend to approach plasma levels.

#### 4. Cl and $\text{HCO}_3$ Concentrations

Contrary to Wheeler and Ramos' findings, the tendency of Cl and  $\text{HCO}_3$  concentrations to rise (or if the hypothetical electrolyte fraction is calculated, to equalize at 75 mEq/L) was found in all cases of continuous bile drainage with and without bile acid replacement. Low concentrations were found only in common duct bile where those of Cl and  $\text{HCO}_3$  of the electrolyte fraction approach plasma levels. No direct relation of these concentrations to the rate of biliary flow has been observed.

#### 5. "Stop Flow" Studies

When flows were stopped in seven experiments for 5-12 minutes and multiple small (0.6-1.0 ml) specimens were examined (Table III) high Cl and  $\text{HCO}_3$  concentrations of remarkable uniformity were encountered. With administration of secretin (Table IV) the rate of flow was markedly increased suggesting secretion against pressure but Cl and  $\text{HCO}_3$  concentrations were similar. Following administration of Diamox (Table V) the only difference noted was the markedly higher concentration of Cl and concomitantly lower concentration

TABLE III  
*Stop Flow Study with Bile Acid Replacement\**  
(Representative Dog Experiment No. 2060-6)

	Pre-study specimen	Consecutive collections of 0.6 ml										
Cl in mEq/L	65	59.0	53.0	47.5	49.0	53.0	50.8	53.0	47.5	55.0	53.0	
HCO <sub>3</sub> in mEq/L	29	40.0	50.0	48.0	50.0	50.0	50.0	50.0	50.0	50.0	56.0	
Flow rate in ml/min	0.2	0.9	0.7	0.52	0.36	0.26	0.3	0.4	0.3	0.25	0.24	

Cl and HCO<sub>3</sub> concentrations show a remarkable consistency with values comparable to those predicted by Wheeler and Ramos (2) for high flow rates. Here the values remain similar despite changing rates.

\* Flow stopped for 10 minutes by raising outflow tube. Bile replaced by intraduodenal instillation of a 10% solution of Na Taurocholate (Dajac) ml for ml following collections. Collections of approximately 0.6 ml.

TABLE IV  
*Stop Flow Study Following Secretin Administration\**  
(Representative Dog Experiment No. 2209-2)

	Consecutive collections of									
	0.9-1.0 ml								5.0 ml	
Cl in mEq/L	40.0	47.8	50.6	50.6	51.3	54.7	55.4		37.3	
HCO <sub>3</sub> in mEq/L	28	44	52	52	42	48	48		32	
Flow rate in ml/minute	2.4	2.4	1.5	1.1	1.8	1.0	0.5		0.5	

Compare these findings with those in Table III. Note the much higher flow rates due to secretin with essentially comparable Cl or HCO<sub>3</sub> concentrations.

\* 70 units pure secretin Vitrum given intravenously one minute prior to stopping flow by raising outflow tube. Bile replaced by continuous infusion of 1% Na Taurocholate (Dajac) solution at 3 ml/minute.

TABLE V  
*Stop Flow Study Following the Administration of Secretin and Diamox\**  
(Representative Dog Experiment No. 2209-1)

	Consecutive collections of									
	0.7 ml	0.8 ml	0.7 ml	0.9 ml	1.2 ml	1.7 ml	1.2 ml	1.6 ml	5.5 ml	1.2 ml
Cl in mEq/L	63.0	84.2	82.1	95.2	88.1	94.3	89.2	81.0	76.5	70.2
HCO <sub>3</sub> in mEq/L	26	36	36	36	40	36	34	32	28	22
Flow rate in ml/minute	2.1	2.4	1.7	1.3	1.2	1.7	1.0	1.2	0.7	0.4

Compare these findings with Table IV and note the high flow rates, higher Cl and lower HCO<sub>3</sub> concentrations.

\* 70 units pure secretin Vitrum and 500 mg Diamox given intravenously one minute prior to stopping flow by raising the outflow tube. Bile replaced by continuous intravenous infusion of a 1% Na Taurocholate (Dajac) solution at 3 ml/minute.



of  $\text{HCO}_3$ . Here too only the first specimen was different due to admixture with a small amount of common duct bile in the collecting tube.

### 6. Studies with Human Volunteers

As seen in Table VI the intravenous administration of secretin, as previously noted by Grossman *et al.* (9), markedly increased flow rates and reduced the concentration of bile acids.  $\text{HCO}_3$  concentrations increased slightly whereas those of Cl did not change significantly. If calculations of the hypothetical

TABLE VI  
*The Composition of Human Bile Following Secretin Stimulation\**

		Flow rate ml/min	Cl mEq/L	$\text{HCO}_3$ mEq/L	Calculated bile acids mEq/L
Pt. R.L.	Pre-secretin.....	0.3	95.3	25	37
	Post-secretin.....	0.75	91.6	36	24
Pt. F.S.	Pre-secretin.....	0.4	101.5	36	
	Post-secretin....	1.4	91.5	40	
Pt. A.D.	Pre-secretin.....	0.4	124.5	18	53.2
	Post-secretin.....	1.2	120.5	43	1.9
Pt. P.M.	Pre-secretin.....	0.2	100	48	
	Post-secretin.....	0.7	96	40	
Pt. D.R.	Pre-secretin.....	0.3	101.3	32	
	Post-secretin.....	1.2	102.8	40	
Pt. I.W.	Pre-secretin.....	0.2	104.6	26	40.0
	Post-secretin.....	1.1	91	28	35.4
Pt. J.C.	Pre-secretin.....	0.4	91	20	45.2
	Post-secretin.....	1.1	89	43	27.4

Note markedly increased flow rates in all cases and reduced concentrations of bile acids in all but one case. Increases of  $\text{HCO}_3$  concentration occurred in all but one case whereas Cl concentrations did not change significantly.

\* Studies were conducted on seven patients following cholecystectomy and common duct exploration. T tubes used in these cases carried a balloon on the distal limb permitting closure of the distal duct during studies to prevent pancreatic reflux. Studies performed between one and two weeks following the operation. Specimen were collected prior to and for 20 minutes following the intravenous administration of 1.0 units/kg of secretin Vitrum.

electrolyte fraction are applied to these findings it is readily seen that the tendency of the concentrations of Cl and  $\text{HCO}_3$  to approach 75 mEq/L is by far not as marked as in the bile of dogs.

### DISCUSSION

Attempts to elucidate the exact mode of bile formation have not only been hampered by technical difficulties in observing bile flow and collecting specimens without altering physiological mechanisms but also by species differences between the animals used (10) and by the multitude of factors involved.

Although the method utilized by Wheeler and Ramos (2) approaches physiological conditions, the objection may be raised that continuous flow of bile in the

cholecystectomized dog is not equivalent to normal collection of bile in the biliary ducts with intermittent ejection into the duodenum. It must be assumed that possible major influences on bile during its passage "downstream" may be minimized during these experiments. In addition pressure differences at the major site of formation of bile may also be of importance.

The attempt to circumvent these objections by utilizing "Stop Flow" studies with the hope of finding different sites of influence on biliary constituents failed. While the uniformity of electrolyte concentrations may be due to rapid diffusion it may also point to a site of secretion of Class A substances farther downstream than previously assumed.

Since the mucosa of the intestinal tract is capable of active electrolyte transfer involving particularly  $\text{HCO}_3^-$  (11), the developmentally related bile ducts may also function in this manner. Rous and McMaster (12) showed that obstructed ducts were capable of secreting clear, slightly alkaline fluid. Ravdin *et al.* (13) studied gallbladder function and proved the possibility of active electrolyte transfer through its mucosa. Herman *et al.* (14) showed that the concentration of  $\text{HCO}_3^-$  in the guinea pig gallbladder was lowered in the presence of bile acids and invoked the Donnan effect to explain this finding. These data, combined with our own, point to important active, although far from completely defined, influences of the bile ducts on the final composition of bile under physiological conditions.

Since Dreiling and Janowitz (15) advanced the hypothesis that the mechanism of pancreatic secretion is best explained by the formation of a primary fluid consisting of  $\text{NaHCO}_3$ , isotonic with blood and undergoing alteration in the ductular system, the above data may be assumed to further point to an analogy between pancreatic and biliary secretion. However, it must be pointed out that volume and  $\text{HCO}_3^-$  concentration differences between pancreatic fluid and the hypothetical biliary electrolyte fraction remain unexplained. Although endogenous secretin may well act under physiological conditions, its mode and site of action in pancreas and liver remain obscure. It is possible that its activity differs at different sites of the biliary system, thereby explaining the absence of an antagonistic effect of Diamox as observed in the pancreas.

In an attempt to elucidate sites of secretion, recent anatomical and histological studies provided additional important data. Ashworth and Sanders (16) showed the presence of two pathways from the liver cell, one directly from the cell through a membrane carrying microvilli suggesting an energy consuming transfer mechanism and one through narrow channels, capable of ultrafiltration. These studies were conducted on livers from rats and humans. Edlund and Hanzon (17) showed that branches of the intercellular bile capillaries entered into the sinusoidal wall. In addition Elias' (18) studies support the concept of the presence of a peribiliary plexus supplying biliary ductular cells. Evidence of preferential ductular secretion of BSP during perfusion studies in dogs has been presented (19). Singer *et al.* (20) showed differing rates of secretion of biliary components in case of proliferation of ductular cells following the administration of ethionine to rats.

The presence of preferential sites of secretion of different substances points to an additional difficulty of interpreting the described experiments by Wheeler and Ramos (2). While continuous intravenous infusion of bile acids seems to circumvent the interruption of the enterohepatic circulation, it may alter the preferential route of action of bile acids. Although Schiff (7) already pointed out that the enterohepatic circulation was active despite ligation of the portal vein, Foster, Hooper and Whipple (21) showed that less than half the normal amount of bile acids was secreted in the presence of an Eck fistula. We were able to confirm these findings in the case of one dog in whom, in addition to the cholecystectomy, and placement of a Thomas duodenal fistula, and end-to-side portal vein-vena cava anastomosis had been performed. The replacement of bile acids intraduodenally might therefore be a more physiological route. The possible action of vascular and nervous influences on bile secretion (22) during intravenous bile acid replacement must also be considered. It is obvious that further studies are needed in order to define the exact function of the described sites of secretion.

While our findings with human volunteers confirm the findings of Grossman *et al.* (9) and show, to a diminished degree, the electrolyte changes observed by Wheeler and Ramos in dogs, they are admittedly unphysiological, having been conducted on recently operated patients. It should however be noted that the presence of a balloon blocking the distal limb prevented the reflux of pancreatic fluid and thereby again proved the choleretic action of secretin. Here, too, we are unable, despite obvious response to secretin of the electrolyte concentrations, to establish the presence of an electrolyte fraction analogous to pancreatic secretion.

#### CONCLUSION

Despite much factual evidence, the exact mechanism of bile formation and flow regulation has as yet not been satisfactorily explained.

Our studies confirm Wheeler and Ramos' (2) findings with exception of their reported direct relationship between the rate of biliary flow and the concentration of  $\text{Cl}$  and  $\text{HCO}_3$ . While analogy between pancreatic and biliary secretion is suggested, several important differences between pancreatic fluid and the hypothetical biliary electrolyte fraction are pointed out. Two important theoretical objections to the physiological state of these experiments are also raised and the need for further studies is stressed.

In addition, our studies suggest that the ductal mucosa may play a hitherto unstressed role in the formation of bile prior to its arrival in the duodenum.

#### ACKNOWLEDGMENTS

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# Percutaneous Transfemoral Renal Arteriography

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Our results, in the past several months, with aortography and selective renal angiography performed by the percutaneous transfemoral route have been most satisfactory. We feel that this method of outlining the abdominal aorta and its branches deserves wider application and it is the purpose of this paper to describe our recent experience. Some of the advantages of the retrograde femoral catheter method will be mentioned, and representative films will be demonstrated.

## METHODS

Our technique, with minor modifications, is essentially that introduced by Seldinger (1) and recently described in detail by Miller *et al.* (2).

No preparation is usually necessary, but the patient should have no food for 12 hours prior to study. With the patient supine, a percutaneous puncture of the right femoral artery is done under local anesthesia. A stainless steel guide wire is introduced well up into the iliac artery. The cannula is then withdrawn, while the guide wire is left in place. Local digital pressure is applied to prevent bleeding until a radiopaque polyethylene catheter is threaded over the guide wire, and passed to the level of the renal arteries. For aortography we use a straight catheter with several side-holes, while for selective angiography a pre-shaped catheter, with a single terminal hole is introduced (3). These two catheters are interchangeable in the course of the procedure so that both aortography and selective arteriography can be performed as a single examination.

For aortography we use 25 cc of either 50% hypaque or Angio-Conray, depending on the size of the patient, introduced with a Gidlund pressure injector. For selective renal arteriography, 5-6 cc of either 50% or 75% hypaque are rapidly injected by hand. In both cases, multiple exposures are taken with a Schönander rapid film changer.

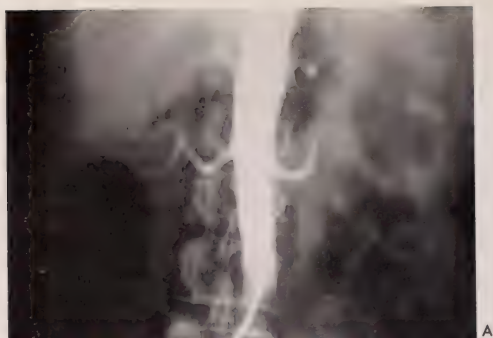
After satisfactory films have been obtained, the catheter is removed from the femoral artery. Direct digital pressure is applied until hemostasis is complete. No pressure dressing is usually necessary.

The patient remains in bed for approximately 12 hours following the procedure, and then may be up and about without restriction. The usual time required for an average procedure is about one hour.

## RESULTS

During a seven-month period, 56 procedures were performed in 54 patients using the method described above. Thirty-three patients had aortography alone. We did selective renal arteriography on 17 patients and 4 patients had aortography and simultaneous selective catheterization of one renal vein. The studies

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A



B



C



have been of uniformly good quality and in virtually all cases have been diagnostic. We were unable to pass the catheter beyond the aortic bifurcation in only one patient.

The use of a rapid film changer enables us to get several exposures in the 1 to 2 seconds the arteries are filled with contrast (Fig. 1). During this "arterial phase" various abnormalities can be demonstrated.

Figure 2 shows a normal left renal artery, and right renal artery stenosis with post-stenotic dilatation. Figure 3 demonstrates a very small left renal artery, partially obscured by overlying splenic vessels, and severe right renal artery stenosis with post-stenotic dilatation. Figure 4 shows fibromuscular hyperplasia of the right renal artery in a 20 year old girl with severe hypertension. Figure 5 shows an accessory artery to the lower pole of the left kidney, an anomaly that occurs in approximately 15% of normal patients. Figure 6 shows typical "puddling" in the upper pole of the right kidney, consistent with right renal tumor.

The arterial phase is immediately followed by the nephrogram phase. As the contrast material localizes within the renal parenchyma the renal outlines are well seen (Fig. 7). Additional information about renal size, contour and position is obtained at this time. Figure 8 shows an atrophic pyelonephritis, and Figure 9 shows a hypoplastic right kidney.

Complications of retrograde femoral aortography have been described in the literature (4, 5). We have had one thus far. A 39 year old Puerto Rican male with sickle cell disease had an uneventful selective catheterization of his left renal artery. Three weeks later an occlusion of the iliac artery requiring aorto-femoral bypass was demonstrated.

We have had relatively little trouble with hematomas following the procedure, although four patients have needed some pressure over the site of puncture after they returned to their rooms. One patient with severe hypertensive cardiovascular disease suffered a myocardial infarction immediately following aortography, but recovered uneventfully. While it is conceivable that this was related to the procedure, similar patients have undergone aortography without complication.

#### DISCUSSION

In our hands, renal angiography by the percutaneous femoral route has certain advantages. The patients can lie comfortably on their backs throughout the course of the procedure. There is no blind puncture of the aorta, and intramural injection of contrast is quite unlikely. After removal of the needle, bleeding is easily controlled with direct pressure. With puncture of the artery below the inguinal ligament, any bleeding which may occur is superficial and can be detected promptly.

The most important advantage of this technique lies in the quality of the

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FIG. 1A. Normal renal arteriogram. Early arterial phase.

FIG. 1B. Normal renal arteriogram. Mid-arterial phase.

FIG. 1C. Normal renal arteriogram. Late arterial phase.

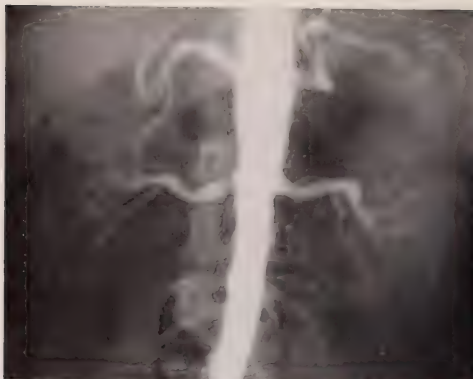


FIG. 2. Aortogram. Right renal artery stenosis with post-stenotic dilatation.



FIG. 3. Aortogram. Small left renal artery behind splenic and superior mesenteric arteries and severe right renal artery stenosis.

films which are obtained. We can routinely expect informative studies. Since the catheter can be placed at any desired level, and the patient placed in any desired position after insertion of the catheter, adequate delineation of the area in question can be virtually assured. Since the right renal artery often originates somewhat anteriorly we have not infrequently had to use a right oblique decubitus position in order to visualize its origin. This is well shown in Figure 10. The right oblique decubitus shows a moderate stenosis at the origin of the right renal artery, that is not all apparent on the antero-posterior projection.

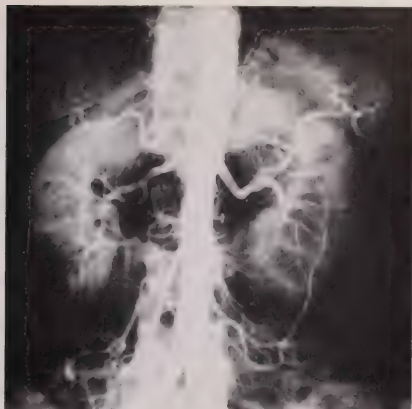


FIG. 4. Aortogram. Fibromuscular hyperplasia in the distal one-third of the main right renal artery and the early portion of the anterior branch.



FIG. 5. Aortogram. Main left renal artery originating at first lumbar interspace. Accessory left renal artery originating at second lumbar interspace.

The introduction of a preshaped catheter also makes possible the selective catheterization of many of the branches of the abdominal aorta. The injection of a small amount of contrast material directly into the renal artery does away with the problems of overlying intestinal vessels that can be so troublesome with simple aortography. It also allows for superior delineation of both the arterial and nephrogram phases by making it possible to deliver a more concentrated



FIG. 6. Aortogram. Mass in the upper pole of right kidney with "puddling" consistent with right renal tumor. Confirmed surgically.

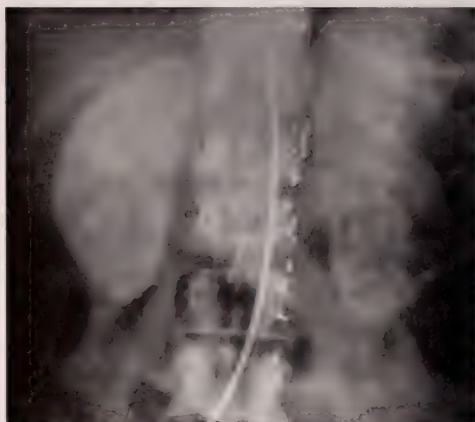


FIG. 7. Aortogram. Normal nephrogram.

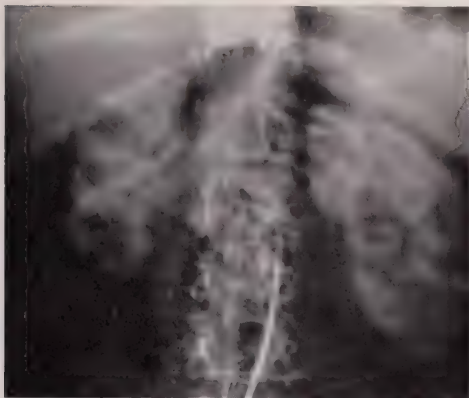


FIG. 8. Aortogram. Nephrogram demonstrating atrophic right pyelonephritis.



FIG. 9. Aortogram. Nephrogram demonstrating hypoplastic right kidney.

bolus of contrast material in a shorter period of time (Fig. 11). Since tubular capacity is exceeded, it is not unusual to visualize the renal vein with a study of this sort (Fig. 12).

We have found selective renal arteriography to be most useful in the differentiation of renal tumors and renal cysts (Figs. 13, 14). There has been excellent correlation between the angiographic representations and the surgical specimens. By performing a femoral vein puncture, the renal veins can be out-

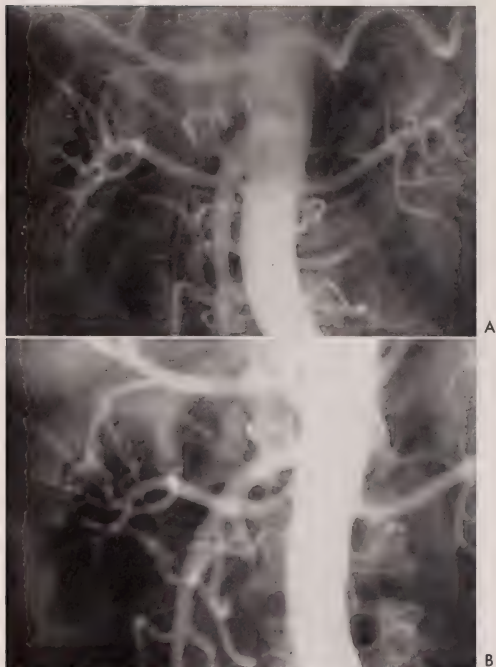


FIG. 10A. Aortogram. Origin of right renal artery obscured in antero-posterior projection, by overlying superior mesenteric artery and aorta.

FIG. 10B. Same patient as Figure 10A. Right oblique decubitus projection brings out mild stenosis at origin of right renal artery.



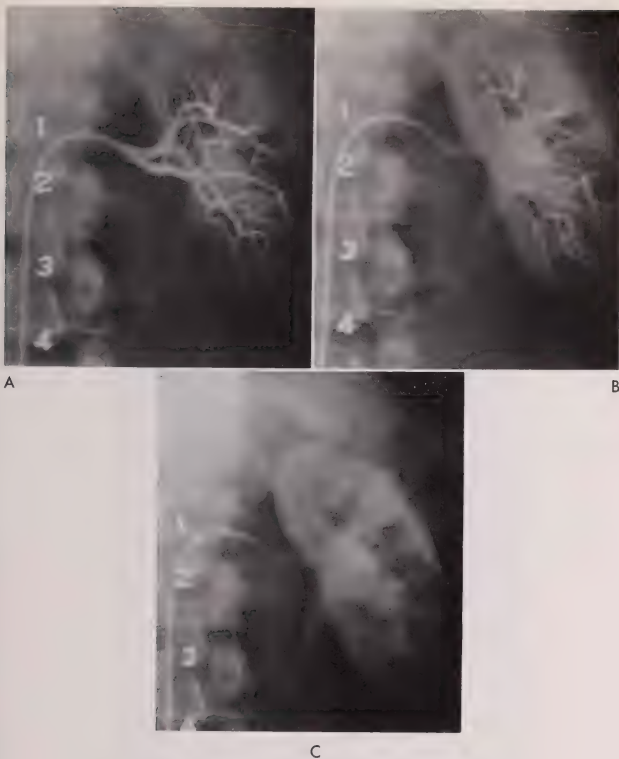


FIG. 11A. Selective left renal arteriogram. Early arterial phase.  
FIG. 11B. Selective left renal arteriogram. Late arterial phase.  
FIG. 11C. Selective left renal arteriogram. Nephrogram phase.

lined as well (Fig. 15). While we have been most interested in the renal vessels, we have also catheterized other vessels. Figure 16 demonstrates injection of the celiac artery in a patient with carcinoid syndrome and hepatic metastases.

Clearance studies have shown that direct renal arterial injection of contrast material results in only transient changes in renal function (6). There is no pain with the injection, and in our limited experience there have been no infections. With the technique employed, we have had no aneurysms, or arteriovenous fistulae. Femoral artery thrombosis always presents a potential threat, and we have a vascular surgeon alerted in case this should occur.



Fig. 12. Selective left renal arteriogram. Visualization of renal vein.

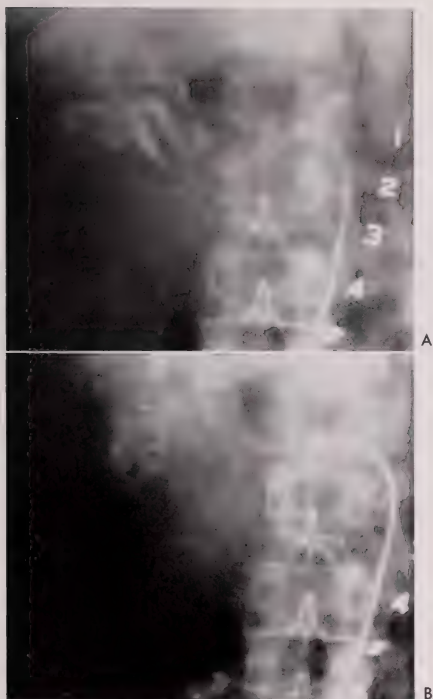


FIG. 13A. Selective right renal arteriogram. Mid-arterial phase with anomalous vessels consistent with right renal tumor.

FIG. 13B. Selective right renal arteriogram. Same patient as 13A. Late arterial phase shows typical "puddling." Presence of tumor confirmed surgically.

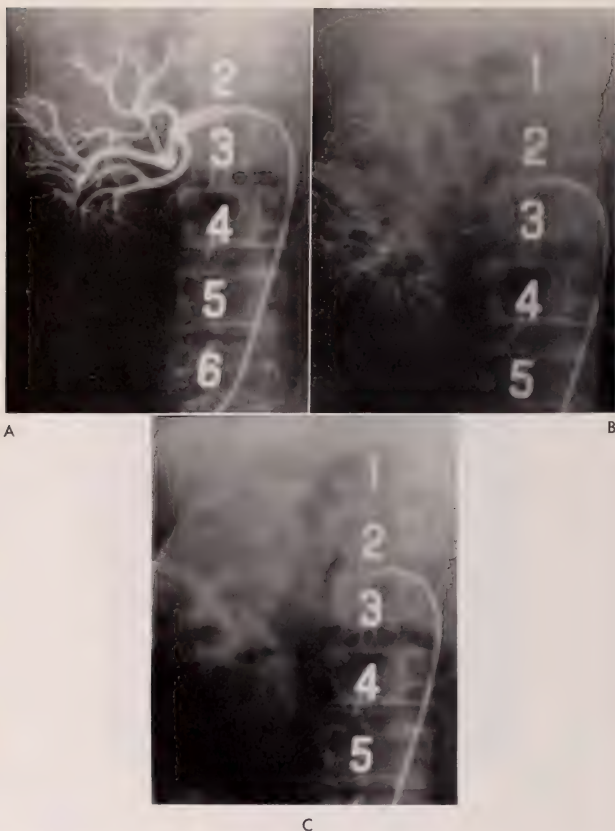


FIG. 14A. Selective right renal arteriogram. Mid-arterial phase. "Spidery" vessels overlying renal cysts beginning to visualize.

FIG. 14B. Selective right renal arteriogram. Late arterial phase. Further delineation of attenuated arteries. Note absence of abnormal tumor vessels.

FIG. 14C. Selective right renal arteriogram. Nephrogram phase. Multiple radiolucent areas consistent with renal cysts. Note absence of tumor staining or puddling.



FIG. 15. Selective right renal venogram.



FIG. 16. Selective catheterization of celiac artery in patient with huge liver secondary to carcinoid hepatic metastases.

#### SUMMARY AND CONCLUSIONS

In the past several months we have performed 56 percutaneous femoral artery catheterizations for the purpose of aortography and selective renal angiography. We feel that this is an excellent method for outlining the abdominal aorta and its

branches. We are uniformly able to obtain excellent visualization of these vessels. One serious complication has been observed.

#### ACKNOWLEDGMENT

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# Keloids: A New Treatment

## Three Case Reports\*

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In spite of the fact that keloids have been known for a long time, and that they have probably occurred in surgical wounds as long as surgeons have been at work, there has been very little progress in the unraveling of the mystery of their causation or in their effective treatment.

Alibert (1) in 1806 was the first to describe the appearance of keloid scars in humans. Since then, there have been further reports of their appearance in horses, cattle and dogs (2). Their etiology is still as uncertain as when they were first described, but their association with certain features, such as pigmentation, the amount of collagen in the skin (3), special sites in the body, slow-healing wounds, certain endocrine states, buried keratin (4, 5), some families and certain races, has been well documented. The only incontrovertible features appear to be that they are associated with proliferation and hyperactivity of the fibroblasts in a healing wound and that this results in excessive laying down of collagen fibers.

The treatments suggested have been equally numerous. Surgical excision and abrasion, the use of caustics, eradication of the lesions by extreme heat (cautery) or cold (CO<sub>2</sub> snow) is variously practiced. The recurrence rate is high. Treatment by means of radiotherapy (6) has had some success, as has the local or systemic administration of a variety of steroids (7, 8). These have been used alone or combined with surgery. Probably the best results obtained have been with surgical excision followed by x-ray therapy, and this is the method in use at most centers, *faute de mieux*. By means of this combined therapy a 61 per cent success rate has been reported by Cosman, Crikelair, *et al.* (2). This is the usual experience of most surgeons treating keloids.

We have been dissatisfied with the limited chance of success to be expected from the therapeutic agents in use at the present time. It seemed reasonable to expect that if multiplication of cells could be reduced by antimetabolic drugs the formation of a keloid would be prevented. This hypothesis was partly confirmed when it was found that a keloid, which had been present for many years on the leg of a patient, began to undergo spontaneous resolution after the patient's pelvis was perfused with a cytotoxic drug for a pelvic carcinoma (9).

It was decided to apply the effect of the chemical antimetabolic agents to the treatment of keloid scars. We have used, in the first place, one of the less toxic of the drugs, and applied it only to the surface of the scar resulting from

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the excision of a keloid. In this way, some would be absorbed at the point where its action is needed without any appreciable systemic effect. The quantity used (50-100 milligrams of a 0.5% concentration of ThioTEPA in a base of aqueous cream) could hardly be expected to produce any systemic effects, even if it were totally absorbed. However as the teratogenic effect of these drugs has not been explored, it was deemed wise to treat only patients who were not pregnant, and to carry out routine estimations of the hemoglobin and white blood cell counts.

#### CASE NO. 1

D.L. is a 16 year old girl who has been an epileptic for the last five years. During an attack in July 1962 she burnt the right arm on the lateral side of the antecubital fossa. This burn took five or six weeks to heal. A keloid developed which has been unchanged during the six months before admission. It was 3.5 cm in length and 1 cm in width, lying in a horizontal band just lateral to the antecubital fossa.

On October 16, 1963, the keloid was excised and a Wolfe graft from the right iliac fossa was sutured into its place. The Wolfe graft "took" well, but there were two areas at the periphery which had not healed (*i.e.*, 80% of the Wolfe graft healed). It was decided to wait before applying ThioTEPA in order to allow complete healing to take place. However, three weeks after excision of the keloid, there were signs of recurrence of the keloid in the scar surrounding the Wolfe graft. It was becoming red, raised, thickened and tender. Accordingly the first application of ThioTEPA (0.1%) was made. Little change was seen after this, or after the second or third applications (on November 11, 1963, and November 14, 1963). The keloid continued to grow. It was therefore decided to use the drug in a concentration of 0.5%. She had, in all, ten applications of ThioTEPA at half-weekly intervals, the first three being 0.1% and from November 18, 1963, onwards 0.5%. There was no increase in the size of the keloid after November 18, 1963.

Two months later it was still static, and three months later the lesion was becoming softer and flatter. When last seen it was still static, six months following excision.

#### CASE NO. 2

M.M. is a 17 year old girl who developed a keloid on the point of the left shoulder four years before admission. It followed a B.C.G. vaccination. The keloid measured 1.5 cm in diameter and was circular. It was raised by about 5 mm from the surrounding skin (Fig. 1).

On November 1, 1963, the keloid was excised in a horizontally placed ellipse and the skin closed by a continuous subcuticular nylon suture. The wound healed without incident and seventeen days after excision, *i.e.*, on November 18, 1963, the first application of 0.5% ThioTEPA ointment was begun. She had, in all, ten applications of 0.5% ThioTEPA ointment at half-weekly intervals. The scar was seen to spread during the first two weeks of

the treatment. During the last week of the treatment, some pigmentation was noted in the area being treated. After completion of the treatment the area around the scar was reddish and crusted. There was no sign of recurrence of the keloid. The crusting disappeared by January 14, 1964, *i.e.*, four weeks after the last application. Photographs taken on January 14, 1964, show a flat, healed, spread scar. (Fig. 2). There has been no sign of recurrence of the keloid when last seen (February 18, 1964).

## CASE NO. 3

M.D., a 22 year old married white woman, was involved in a car accident three years ago. She was in the left front passenger seat and sustained a

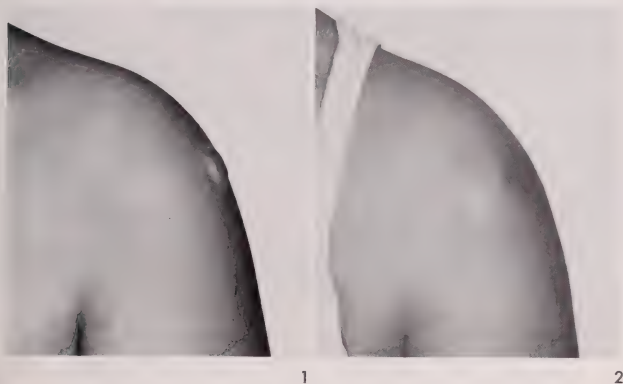


FIG. 1. Case 2. Keloid on shoulder before excision.

FIG. 2. Case 2. Two months after excision, followed by topical chemotherapy.

fractured right femur and cuts on the left upper arm. These were sutured at another hospital.

She was first seen complaining of large keloidal scars of the left upper arm in January 1962. They were still developing and it was decided to leave them to mature. They were seen at six monthly intervals and continued to grow and mature (Fig. 3).

On November 15, 1963, under general anesthesia, the two keloids on the back of the arm were excised. The skin was undermined and closed primarily using continuous intradermal sutures.

On November 28, 1963, *i.e.*, thirteen days after the operation, she was treated by the first application of ThioTEPA ointment (0.5%), before the scars had completely healed. Two further applications were given, on December 2, 1963, and December 5, 1963. In all, she received three applications of 0.5% Thio-

TEPA ointment at half-weekly intervals within eight days. By the time of the last application all the wounds had healed and there was no sign of recurrence. She did not attend for further treatment, owing to an intercurrent upper respiratory infection, which confined her to bed.

She was seen again on February 18, 1964, *i.e.*, three months after excision of the lesion. The scars were somewhat tender but there was only minimal thickening in the upper scar (Fig. 4). The scars measured 3 mm in width at the widest point. There has been no recurrence in the scars up to the present time, five months following excision.

Three cases have been treated so far, and in these three there have been no



3



4

FIG. 3. Case 3. Keloids on upper arm before excision.

FIG. 4. The same patient (Case 3) some five and a half months following excision and topical chemotherapy.

recurrences of the keloid to date, some seven months after the excision. It is realized that this number is not conclusive proof of the efficacy of the treatment, but the success in the patients treated (none of the keloids have recurred or increased in size after the application of 0.5% ointment) without any side effects has encouraged us to continue the investigation with a larger series of patients. The best drug and the best dosage schedule yet remain to be worked out. This will be done in the larger series.

#### SUMMARY

The etiology of keloid scars is briefly discussed and the variety of treatments for this condition is outlined. A new method of treatment is described and three cases are presented. These cases were treated by excision of the keloid, followed by topical application of ThioTEPA to the resulting scars. In none of the three cases has the keloid recurred after the use of the ointment

in a 0.5% concentration. One would normally hesitate to present yet another treatment for this common condition, but the success obtained in this preliminary series is encouraging.

#### ACKNOWLEDGMENTS

I should like to thank Mr. R. J. V. Battle for his kind help and interest in this preliminary study and Mr. G. Westbury and Mr. P. Clarkson for much valuable advice.

The photographs were taken by Mr. E. B. Ferrill. They are reproduced here by the kind permission of Queen Mary's Hospital, Roehampton (London, England).

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# Curling's Ulcer: A Case Report

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## INTRODUCTION

Curling's ulcer is the common term applied to a gastrointestinal ulcer which manifests itself in a severely burned patient.

Historically, Curling was not the first author to describe this condition. He was preceded by Swan (1) and Long (2). Curling's report appeared in 1842 and can be read in the original form in the New York Academy of Medicine Library. His article (3) described a bleeding duodenal ulcer which appeared in a severely burned patient. Several years later, he described the perforation of a duodenal ulcer in another severely burned individual (4). Today the term Curling's ulcer is not limited to the duodenum but refers to any ulceration which occurs in the gastrointestinal tract of a burned patient.

## DISCUSSION

Approximately one hundred and twenty-seven cases of Curling's ulcer have been reported to date. This pathologic entity almost certainly occurs more frequently than these figures indicate. The relative paucity of reports probably derives from: 1) the lack of routine thorough post mortem examinations in all burned victims, and 2) the failure to recognize the condition in those who survive their burns without complications or symptoms of ulcer.

In 600 burned patients studied at the Brooke Army Medical Burn Center (5) in San Antonio, Texas, 5 patients of 20 who died had proven ulcers. One patient had massive bleeding commencing on the tenth post-burn day. At post mortem examination, the patient had three acute duodenal ulcers and one superficial gastric ulcer. A second patient started bleeding after forty-eight hours and then perforated a gastric ulcer prior to death. The third patient at no time showed clinical evidence of bleeding. He died on the thirteenth day, exhibiting a large duodenal ulcer at post mortem examination. The fourth patient died on the sixth post-burn day from acute ulcers of the stomach and the duodenum. The fifth patient sustained 85% body burn, died within 24 hours and demonstrated superficial ulcers of the duodenum and the ileum. Silent ulcers therefore do occur in this condition.

This same group later reported 24 gastrointestinal bleeders in burned patients, of their own and 17 authenticated cases in the literature. They found a total of 20 proven ulcers in the duodenum, 9 in the stomach, 5 in the esophagus, and one in the terminal ileum.

According to Sevitt (6), when the bleeding occurs early after the burn, it is probably due to gastric erosion. When it occurs after several days, it is probably duodenal in origin. Sevitt states that perforation does not occur

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for at least several weeks after the burn, usually occurring approximately four weeks later.

Histologically, the ulcers which appear in the stomach are multiple superficial erosions. When ulceration occurs in the duodenum, the ulcers are usually present in the first portion, on the posterior wall, and are usually deep, penetrating into the pancreas and into the adjacent pancreaticoduodenal vasculature. Curling's ulcer may also be multiple in situation as well as number. In the remaining bowel, the terminal portion of the ileum is usually more involved than the jejunum and usually shows evidence of hemorrhagic congestion. Cecal and ascending colonic involvement have also been reported.

There appears to be no agreement in the literature as to the pathogenesis of this entity. Various mechanisms postulated include: 1) the effect of toxins, 2) increased secretion of histamine, 3) increased gastric acid secretion, 4) adrenal failure, 5) fat embolization, 6) septic embolization, 7) infection, 8) thrombosis, 9) capillary stasis, 10) dehydration, 11) hemoconcentration, 12) shock, and 13) neurogenic reflexes. A complete discussion of pathogenesis is beyond the scope of this report.

Friesen (7) showed first that stimulation of gastric secretion in extensively burned dogs by the administration of histamine in beeswax greatly increased the frequency of ulceration and secondly, that subtotal gastrectomy of animals later subjected to burning plus histamine administration prevented duodenal ulceration. He also established that post-burn hemoconcentration in dogs was closely associated with the development of gastric and duodenal congestion, as well as erosions and peptic ulceration when gastric secretion was stimulated by histamine. He noted that when hemoconcentration was prevented by transfusion of plasma, gastroduodenal lesions failed to appear. In unburned dogs, hemoconcentrated without dehydration or oligemia by electrophoresis (withdrawal of blood followed by re-infusion of cells), the administration of histamine regularly produced mucosal congestion and ulceration. When hemoconcentration was not produced by this experimental method, histamine injection failed to induce peptic ulceration.

Some of the authors (8) of recent articles state that Curling's ulcers are probably a form of stress ulcer following major surgery or major trauma and that ulceration is probably the result of increased adrenocortical activity.

At The Mount Sinai Hospital during the ten-year period 1952 to and including 1961, 253 patients were admitted because of burns. It is of note that none of these patients presented evidence of any gastrointestinal bleeding. The present report is our first experience with a proven Curling's ulcer.

#### CASE REPORT

Patient M.Q., a 5 year old female, was admitted for the first time to The Mount Sinai Hospital on March 13, 1963, at 3 A.M. She was brought by the police after being involved in a fire in her home. Her mother and brother were admitted to another hospital. By the "rule of 9 of Wallace," it was estimated on admission that she sustained a body burn of at least 50% which

included first and second degree burns of her right and left legs, her right hand, left arm, left side of chest, neck, face and lower anterior abdominal wall. Her admission weight was 16 kilogram and her admission hematocrit was 44%.

Initial therapy consisted of whole blood, plasma, saline, antibiotics (4 million units of penicillin and one gram of chloromycetin in 24 hours), tetanus toxoid, sedation, cultures of throat and burned area, isolation in sterile surroundings, a cut down in the arm, a Foley catheter with accurate measurement of urinary output, and daily evaluation of the electrolytes, blood urea nitrogen, hematocrit, complete blood counts, etc.

After nine hours of observation, it was apparent that the burns were second and third degree rather than first and second. The hematocrit had risen to 47%. The urinary output was 80 cc per hour.

At the end of the first 24 hour period, the child had received intravenously 2900 cc of 5% glucose in saline, 250 cc of plasma, and 250 cc of whole blood for a total fluid intake of 3400 cc. Her blood pressure was 110/90 mm Hg, the pulse rate 120 per minute, and respirations were 20 to 30 per minute. Her urinary output was 980 cc in 24 hours with a specific gravity of 1020. The temperature rose to 104.4 degrees F and the hematocrit to 56%. The blood urea nitrogen was 19 mg%, sodium 144 mEq/L, chloride 100 mEq/L, and potassium 4.4 mEq/L.

It was deemed impossible to apply skin grafts to this patient's burned areas at this time. The child progressively improved and appeared comfortable. On the fourth post-burn day, the temperature was 102 degrees F, the hematocrit was 38%, the 24 hour urinary output was 900 cc with an oral intake of milk of 210 cc and a total 24 hour fluid intake of 1750 ml. The cultures of the burn wounds grew *E. coli*, *Staphylococcus aureus* and *Enterococcus*. There was a trace of bile in the urine, urobilinogen concentration was 1:80. The hemoglobin was 13.6 grams and the white blood cell count 36,500 per cu mm. The child was taken out of oxygen, placed on a Stryker frame, and started on a soft diet.

Approximately 114 hours after admission, the patient vomited blood clots and bloody mucus for the first time. However, the general status was still satisfactory, the burns were drying, and the patient was taking, without complaint, egg-nogs, milk, malteds, etc., by mouth.

Twenty-four hours later, on the sixth burn day, the child again vomited bright red blood. This time, she appeared to be in shock with a pulse rate of 180 per minute, a blood pressure of 110 mm Hg, and a hematocrit of 11%. A Levine tube inserted into the stomach revealed bloody fluid. Gastrointestinal bleeding continued and gradually increased in spite of ice water lavages. The child remained in poor condition despite frequent blood transfusions. The abdomen gradually became more distended and abdominal x-rays revealed gastrointestinal distention but no definite evidence of G.I. perforation.

On the eighth post-burn day, the child became comatose, her abdomen was distended and there were signs of continuous gastrointestinal hemorrhage. On the tenth post-burn day the child expired.



FIG. 1. Photograph demonstrating a large acute ulcer below the esophagogastric junction and numerous small acute gastric ulcers.

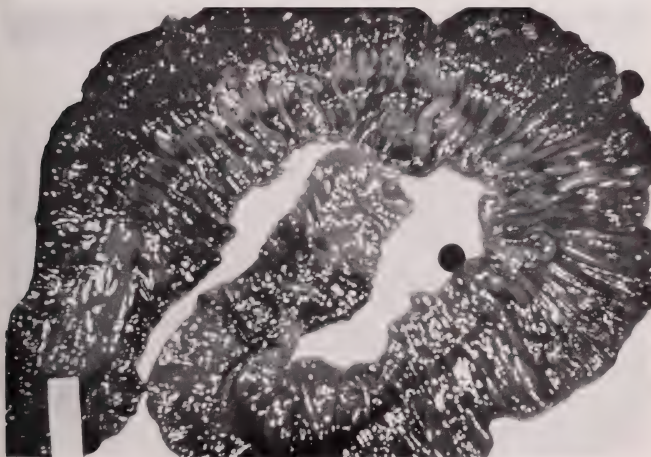


FIG. 2. Photograph demonstrating acute ulceration of the small intestine.

Except for the burned areas, the significant autopsy findings were confined to the gastrointestinal tract. Examination of the esophagus revealed a few erosions of the mucosa but no obvious bleeding points. Just below the esophagogastric junction, there was a large acute ulcer extending one-fourth of the way to the pylorus (Fig. 1). In the gastric mucosa there were at least twelve small acute ulcers, some of which contained fresh blood clots. There were also about two dozen small pinpoint acute ulcers which did not contain blood clots. In the first portion of the duodenum adjacent to the head of the pancreas, there was a large acute ulcer. This ulcer was approximately 3 cm in length and 1½ cm in width. Its base was the head of the pancreas. In the upper part of the small intestine over a distance of approximately one meter were areas of acute ulceration covered by fibrinous hemorrhagic membranes (Fig. 2). The small and large bowel were filled with blood. No mucosal ulcerations were seen in the colon.

In summary, the patient was a severely burned child, age five, with approximately 50% of her body surface sustaining second and third degree burns. She seemed to respond to persistent intensive therapy for the first five days. It was at this time, that signs of gastrointestinal bleeding became evident, gradually increasing in severity and culminating despite all therapy in death on the tenth post-burn day.

#### ACKNOWLEDGMENT

The author wishes to thank Doctor Milton Helpern of the Medical Examiner's Office for his co-operation in obtaining the autopsy findings.

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# The Clinical Use of Intrathecal Methylprednisolone Acetate\* Following Lumbar Puncture†

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The postlumbar puncture syndrome has long been an unavoidable iatrogenic concomitant of diagnostic neurological investigation. Many studies have been carried out in an attempt to define the parameters affecting the postlumbar puncture syndrome and to find an effective method of its prevention and treatment (1). This study was prompted by the failure of the literature to provide a solution to this problem and by an observation that patients with demyelinating disease undergoing multiple lumbar punctures with intrathecal instillations of methylprednisolone acetate rarely complained of postlumbar puncture symptoms (2).

## METHODS AND PROCEDURE

Two hundred patients receiving lumbar punctures admitted to the acute neurology service of The Mount Sinai Hospital were selected for this study. Patients whose level of consciousness was severely impaired, those with subarachnoid hemorrhage, and patients with meningitis were not included. The only requirement for participation in this investigation was the ability of the patient to report reliably on postlumbar puncture symptoms. Depending upon the order of admission, subjects were placed in rotation in one of the four following groups:

Group I. Routine lumbar puncture followed by intrathecal instillation of 10 cc 0.9% saline—50 patients.

Group II. Routine lumbar puncture followed by intrathecal instillation of 40 mg methylprednisolone acetate with polyglycol vehicle in 10 cc 0.9% saline—50 patients.

Group III. Routine lumbar puncture followed by intrathecal instillation of 1 cc. polyglycol vehicle in 10 cc 0.9% saline‡—50 patients.

Group IV. Routine lumbar puncture—50 patients.

All lumbar punctures were performed by one investigator in the following manner: All patients were sitting up at the time of lumbar puncture. The skin was cleansed with antiseptic solution, 2 cc of 1% or 2% procaine hydrochloride was instilled intradermally and subcutaneously, and a number 18 gauge needle was inserted bevel up into the subarachnoid space in the lumbar cistern. The patient was then placed in the lateral decubitus position and manometries were performed if indicated. Samples of 6–10 cc of cerebrospinal fluid were then obtained, the needle removed, and the patient allowed out of bed ad libitum. All

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\* Depo-Medrol, Upjohn Company.

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‡ Kindly provided by Dr. S. Stubbs, Upjohn Company.

patients were examined by the same investigator eight to twenty-four hours following lumbar puncture and daily thereafter. The patients were assured that they would have no symptoms following lumbar puncture, and no patients were aware that they were receiving any injection beyond the routine lumbar puncture. No restriction of activity was recommended and patients were encouraged to be out of bed. The criteria used to determine the presence of the postlumbar puncture syndrome were:

1) Headache intensified in the erect position, with or without accompanying stiff neck, nausea, vomiting, or lightheadedness.

2) Back pain.

3) Pain on neck flexion or straight leg raising.

Each patient was specifically questioned about all of the points enumerated above whether or not they volunteered symptoms.

#### RESULTS

Per cent incidence of headache was used as the most reliable criterion of the postlumbar puncture syndrome since it was invariably present either alone or in combination with other symptoms such as stiff neck, nausea, and lightheadedness. None of the patients had significant pain on straight leg raising following lumbar puncture in the absence of headache. Almost all of the patients when closely questioned admitted to some discomfort in the lumbar region in the area of the needle puncture. However in none of the patients was it disabling or did it require medication.

As can be seen in Table I the patients in each of the four groups are similar in sex and age distribution. The neurological diagnoses were varied but in no one group was there any evident preponderance of a single diagnostic category as compared to the other groups.

As shown in Table I the group of patients receiving methylprednisolone acetate had a statistically significantly lower per cent incidence of headaches following lumbar puncture (Group II) 4%, as compared to each of the other three groups. Patients receiving saline alone (Group I) had a 45% incidence of head-

TABLE I

Group	Sex		Average Age	Per cent Incidence Headache
	Male	Female		
I (Saline)	25	25	52	45
II (Steroid Polyglycol Saline)	24	26	47	4
III (Polyglycol Saline)	24	26	50	42
IV (No Injection)	26	24	47	38



ache, those receiving polyglycol vehicle in saline (Group III) had a 42% incidence of headache, and patients receiving only the lumbar puncture without instillation of any substances (Group IV) had a 38% incidence of headache. There was no significant difference between these latter three groups. The chi square test was used and the level of significance accepted was  $p \leq .05$ . The per cent incidence of postlumbar puncture headache for Group IV is comparable to the figure for average per cent incidence of headache found in the literature (1), while that for Groups I and III is slightly higher.

#### DISCUSSION

Numerous studies have shown a diminished incidence of postlumbar puncture symptoms in patients receiving hydration, bed rest following lumbar puncture, epidural injections, and use of a small gauge needle among other recommendations (1). Many of these suggestions are not practical for diagnostic neurological purposes since manometries cannot be performed adequately with small gauge needles, subsequent neuroradiological procedures render epidural injections undesirable, and prolonged bed rest is impractical in patients undergoing multiple diagnostic tests on a busy neurological service. Many authors have advocated a variety of drugs to treat postlumbar puncture headaches (1), but none of these have been found to be universally successful. Only three studies could be found which investigated the use of steroids in the treatment of postlumbar puncture symptoms. Pfeiffer (3) found prompt remission of postlumbar puncture symptoms in obstetrical patients following oral administration of desoxycorticosterone acetate. Asbell (4) had similar results following intramuscular injection of desoxycorticosterone acetate. Tourtellotte *et al.* (1) failed to show any diminished incidence of postlumbar puncture headache in patients with multiple sclerosis receiving ACTH gel intramuscularly.

As recently as this year a monograph was published (1) citing the available evidence on the treatment of postlumbar puncture headaches and concluding that a cure for this condition had not yet been found. The striking results found with intrathecal methylprednisolone acetate in this study would seem to recommend it as a *prophylactic* agent in the postlumbar puncture syndrome. The few cases in which we have attempted to treat an induced postlumbar puncture headache with methylprednisolone acetate have been totally without success. The advantages of subarachnoid injection of methylprednisolone acetate immediately after lumbar puncture over any other therapeutic program are:

- 1) It prevents the appearance of postlumbar puncture symptoms. In this way the patient is not restricted to bed, other diagnostic tests can be performed without inconvenience to the patient, and the hospital stay is not prolonged by distressing symptoms.

- 2) The systemic effect of the steroid is minimal compared to other modes of administration. This is desirable in diabetic patients and those with ulcers of the gastrointestinal tract, pulmonary or heart disease in whom steroid therapy is potentially hazardous.

- 3) No complications of any kind have been encountered either during hos-

pitalization or subsequently despite a few traumatic lumbar punctures and some multiple punctures. A few patients with demyelinating disease receiving multiple injections of methylprednisolone acetate have had pleocytosis in subsequent cerebrospinal fluid examinations without clinical symptoms.

The mechanism by which subarachnoid injection of steroids prevents postlumbar puncture symptoms is unknown. Whether it is related to their anti-inflammatory properties is speculative, and the role steroids might play in the leakage of cerebrospinal fluid into the epidural space (the most popular theory explaining postlumbar puncture symptoms) (5) is not clear.

#### SUMMARY

Two hundred patients receiving lumbar punctures admitted to an acute neurology service were evenly divided into four groups. In the first group, the patients received equal volume intrathecal instillations of saline, in the second group methylprednisolone acetate and polyglycol vehicle in saline, in the third group polyglycol vehicle in saline only, while the fourth group had nothing injected following lumbar puncture. Examination eight to twenty-four hours and even longer periods after lumbar puncture revealed a statistically significantly diminished incidence of postlumbar puncture symptoms in the group receiving methylprednisolone acetate as compared to the other groups. It is concluded that the intrathecal instillation of methylprednisolone acetate is of substantial clinical value in prevention of symptoms following lumbar punctures.

#### ACKNOWLEDGMENT

The author wishes to express his appreciation to Dr. Morris B. Bender, Chairman, Department of Neurology, for making patient material available, and for his interest and guidance.

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## Radiological Notes

CLAUDE BLOCH, M.D., AND HARVEY M. PECK, M.D.

### CASE NO. 246

A 58 year old male was admitted to the hospital with a five-year history of rectal bleeding. This had been treated intermittently by local injections for "hemorrhoids" with relief of symptoms. The patient was well until two weeks before admission when there was recurrence of rectal bleeding. General physical examination was normal. Digital examination revealed a firm mass filling the rectal ampulla. Sigmoidoscopy revealed a large smooth mass located on the posterior and left lateral walls of the rectum, commencing at the sphincter and extending proximally for three inches. The mucosa was stretched over the mass and in the center there was a large area of shallow ulceration. Biopsy was made which revealed fragments of leiomyoma. Laboratory examination revealed a normal hemogram. A barium enema was performed which showed a large smoothly margined mass originating from the posterior wall of the lowermost part of the rectum (Fig. 1). A large shallow ulceration could be noted on the anterior surface of the mass. There was no evidence of proximal colonic dilatation. The patient underwent a laparotomy for the removal of the tumor, together with a temporary diverting sigmoid colostomy. At operation, a  $7 \times 6 \times 4$  cm rubbery mass was found occupying the posterior wall of the rectum. It was well encapsulated and a shallow ulceration was noted at the apex of the tumor. It weighed 180 grams. Histological examination of the specimen revealed a leiomyoma. The sigmoid colostomy was closed 6 months after the initial operation and the patient had an uneventful recovery.

*Case Report:* LEIOMYOMA OF THE RECTUM.

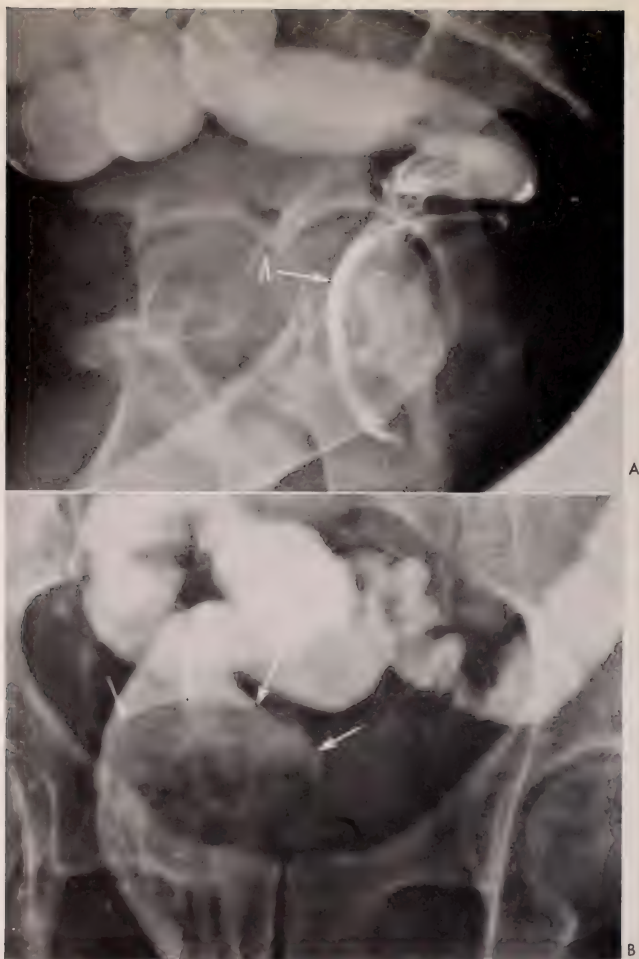
### DISCUSSION

See discussion following Case No. 247.

### CASE NO. 247

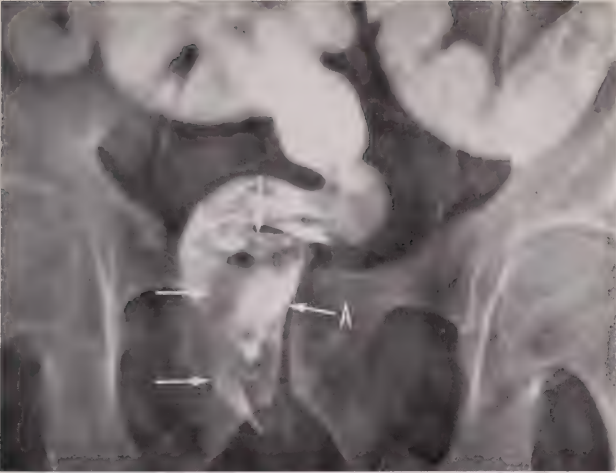
This 63 year old male was admitted to the hospital because of increasing constipation, tenesmus, loss of weight and easy fatigability. There had been no evidence of rectal bleeding or melena. The patient had noted crampy lower abdominal pains without vomiting, relieved by enemas. Physical examination revealed a chronically ill man in no acute distress. The abdomen was distended and a slightly tender fullness was noted in the left lower quadrant. An actual mass could not be delineated even by bimanual palpation. Bowel sounds were active. Laboratory examination revealed a hemoglobin of 9.6 Gm%. Sedimentation rate was 67 mm hr. A barium enema examination was performed which revealed a smoothly defined, lobulated polypoid mass occupying the rectum

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Case 246, Fig. 1A. Lateral view of the rectum and sigmoid during the course of the barium enema reveals a large smooth mass originating from the posterior wall of the lowermost part of the rectum and filling most of the rectal ampulla. The contours are smooth. In the anteriormost position of the mass the barium collects in an amorphous fashion, suggesting the presence of a shallow ulceration (arrow A). There is no proximal dilatation within the sigmoid or remainder of the colon.

Case 246, Fig. 1B. Spot film of the rectum and sigmoid in the frontal projection during the course of filling again reveals the smoothly demarcated mass occupying the rectum. It is seen to originate from the left side of the rectal wall. The superior contour is noted to be smooth (arrows), and there is no evidence of proximal dilatation.



Case 246, Fig. 1C. After incomplete evacuation of barium from the lowermost portion of the colon, the tumor edge is again noted (along arrows), and a large ulceration (arrow A) is now well seen.

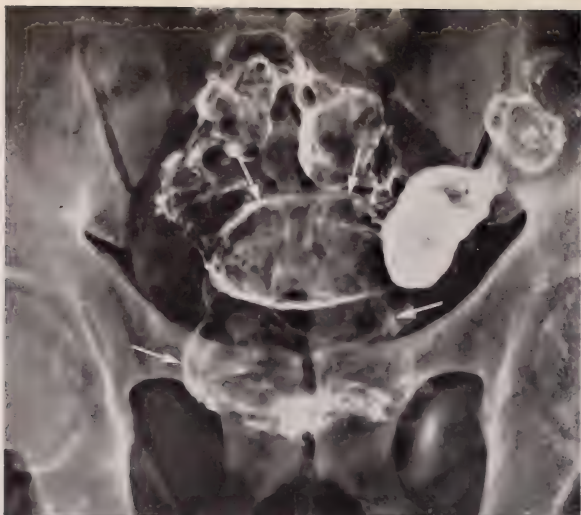
and causing partial retrograde obstruction to the flow of barium (Fig. 1). The mass was noted to measure  $6 \times 8$  cm in diameter and was seen to prolapse considerably into the rectal ampulla during the process of evacuation. Laparotomy was performed, and a grapefruit-sized firm mass occupying the rectum was noted which extended both intraluminally and extracolonicly. Resection was deemed impossible because of infiltration into the left pelvic wall. A diverting colostomy was performed. Biopsy of the tumor mass revealed infiltrating leiomyosarcoma.

*Case Report:* LEIOMYOSARCOMA OF THE RECTUM.

#### DISCUSSION OF CASES NOS. 246 AND 247

In a review of 2525 cases of smooth muscle tumors of the alimentary tract, Skandalakis *et al.* (1) found the incidence of myomatous tumors to be most frequent in the stomach, then in the small bowel, thirdly in the esophagus and lastly in the colon. In all the cases reported in the world literature until 1962, only 144 cases of smooth muscle tumors of the large intestine were found. By far the most common site within the colon is the rectum. It is now thought by some that a number of the myomatous tumors in the rectum represent rhabdomyomas originating in the pararectal tissues rather than from smooth muscle origin (2).





A



B

Case 247, Fig. 1A. There is a large lobulated filling defect occupying the rectosigmoid. The lower edge is poorly made out, but the lateral and upper contours are well seen (along arrows). Very little barium was able to pass proximally to the lesion.

Case 247, Fig. 1B. Air contrast studies again reveal the lobulated mass in the rectosigmoid. The lower contour is seen to better advantage as the tumor has prolapsed into the rectal ampulla and it is thus outlined by air (along arrows). A considerable amount of feces is noted proximal to the lesion.



Myomas of the rectum present roentgenologically as smoothly demarcated intraluminal masses with a broad base. The mucosa is stretched over the lesion and often a central shallow ulceration can be outlined (3). As with other sub-mucosal tumors such as lipomas, these lesions may cause intussusception when they are located in the proximal portions of the colon. Because of the fixed position of the rectum and the lack of a mesentery, only slight tumor prolapse occurs within the rectal region (4).

Most of the patients enter with symptoms of intermittent constipation and diarrhea, mucous and weight loss. Rectal bleeding is present in half the cases. A palpable rectal mass is the rule, being present in 70% of leiomyomas and 90% of sarcomas (5).

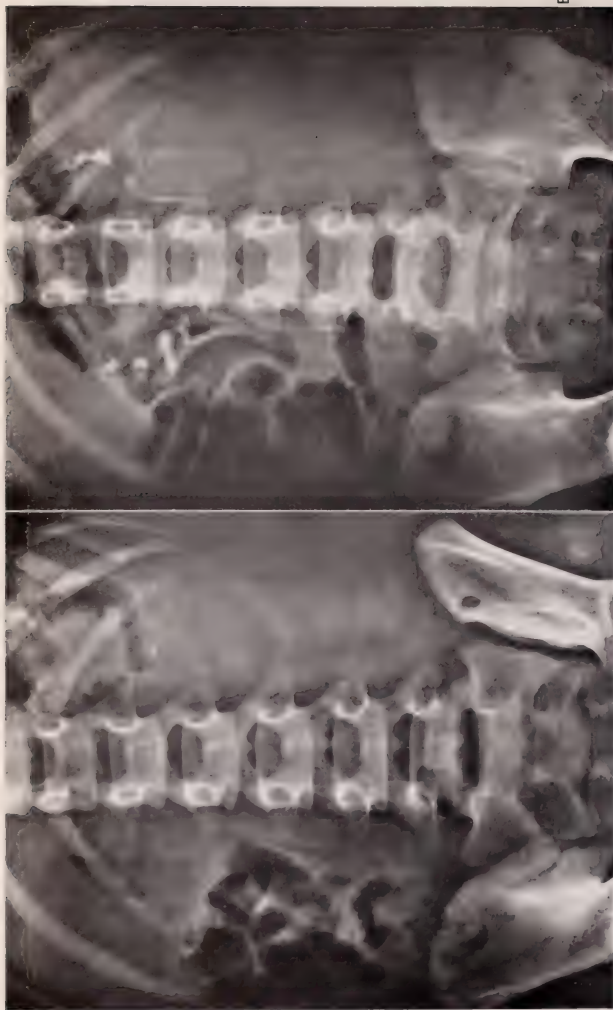
Leiomyosarcomas are usually bigger than the myomas and often extend beyond the confines of the rectal wall. They may attain enormous size. Rarely do they metastasize to nodes, their mode of spread being by direct invasion and infiltration (6). Roentgenologically, they are usually lobulated and ulcerations are more prominent and deeper than in leiomyomas.

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#### CASE NO. 248

This 5 year old girl was admitted to the hospital with a 24 hour history of severe left lower quadrant abdominal pain. Similar, but less intense episodes of pain had occurred intermittently for the past two years, but each had subsided quickly and spontaneously. The present attack was associated with mild diarrhea, but no fever, nausea or vomiting. The patient had undergone an appendectomy two years before the present admission for acute appendicitis. Physical examination revealed an acutely ill child who was guarding her abdomen with flexion of the hips. There was slight dehydration. Generalized voluntary and involuntary muscle spasm was noted on abdominal palpation. There was direct and rebound tenderness in the left lower quadrant. No masses could be palpated. Laboratory examination revealed a normal hemoglobin and a white blood count of 10,500, with a slight shift to the left. Radiographic examination of the abdomen in the prone position revealed absence of gas-filled bowel loops in the left side of the abdomen (Fig. 1). No discrete soft tissue masses could be outlined, and there were no abnormal calcifications.



Case 248, Fig. 1A. Examination of the abdomen in the prone projection reveals absence of intestinal gas in the lower two-thirds of the left side of the abdomen. The only gas filled loops are located in the left upper quadrant and under the left leaf of the diaphragm. No soft tissue masses are identified. There are no abnormal calcifications.

Case 248, Fig. 1B. Examination of the abdomen during the course of an intravenous pyelogram reveals normal renal and psoas structures. Although no soft tissue mass can be outlined, again

Because of persistence of signs and symptoms, laparotomy was performed ten hours after admission. At operation, as the peritoneal cavity was entered, a huge multi-loculated cyst was noted completely filling the left side of the abdomen, extending from the left upper quadrant down towards the midline in the suprapubic region. It was a thin-walled cyst which contained 150 cc of clear colorless fluid. The cyst wall was firmly attached to the leaves of the mesentery in the left upper and lower quadrant and thick adhesions to the transverse colon were lysed. The patient made an uneventful recovery.

*Case Report: MESENTERIC CYST.*

#### DISCUSSION

Mesenteric cysts are rare and may occur at any age, twice as frequently in females as in males (1). They are usually thought to be developmental in origin, but their exact pathogenesis is uncertain and may be related to a low-grade inflammation of the mesentery or to previous surgery (2). They contain clear fluid and are usually thin-walled and multi-loculated. On occasion, they contain calcium within their walls, but this is usually related to previous bleeding in the wall of the cyst or infection (3). So-called "omental cysts" or "lymphatic cysts" are probably very similar in pathogenesis and in content. "Peritoneal cysts" or "localized ascites" usually follow gynecological operations and their pathogenesis is thought to be related to the formation of a loculation when two peritoneal layers are overlapped within the pelvic floor. Roentgenologically, the findings may either be of a sharply delineated soft tissue mass or simply, as in the present case, a generalized displacement of bowel loops without identification of the border of the mass. Calcium may be seen on occasion within the cyst wall. It is important to rule out in the differential diagnosis renal cysts, splenic cysts, pancreatic and duplication cysts.

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#### CASE NO. 249

A 36 year old female sought medical advice because of irregular vaginal bleeding of three weeks' duration. She stated that her menstrual periods were normal until three weeks prior at which time an expected period began. Bleeding continued intermittently and became profuse during the previous twenty-four hours.

General physical examination was normal. Pelvic examination revealed a large exophytic neoplasm which measured approximately 10 cm in diameter and occupied the entire cervix. The lesion obliterated the fornices by extension and involved the upper one-third of the vagina. The medial half of the right parametrium was also infiltrated. The clinical impression was carcinoma of



Case 249, Fig 1. Lateral view of the rectum during the barium enema examination includes the rectum up to the rectosigmoid. The rectum is limited in distensibility but the margins are smooth. There is a mass around the rectum with the bowel separated from the sacral hollow by a distance of 3.5 cm. Distensibility of the bowel seems normal at the rectosigmoid.

the cervix, Stage II. The patient was admitted to the hospital. A biopsy of the lesion was reported as infiltrating squamous carcinoma. The patient was referred for radiotherapy.

Treatment consisted of 250 Kv external beam therapy to six pelvic portals along with 9,100 mg-hrs of radium therapy utilizing vaginal colpostat and intrauterine tandem. The course of radiation represented the standard treatment employed at this institution; it lasted for nine weeks, about one week longer than the average case. One week following the completion of treatment, pelvic examination revealed no evidence of tumor in the cervix, vagina or parametria. An active radiation reaction was apparent. The reaction healed as expected and repeated examinations revealed no evidence of disease. Five months after treatment the patient complained of left sciatica of increasing severity. Eight months after treatment, the rectovaginal septum and both parametria were thickened and nodular, and the rectum was markedly narrowed by surrounding mass. Other complaints now included back pain, rectal pain, frequency and burning on urination and increasing constipation. Barium enema (Fig. 1) showed narrowing of the rectum with smooth margins and



A



B

Case 249, Fig. 2A. Right anterior oblique view of the rectum and rectosigmoid during barium enema examination shows limited distensibility from the rectosigmoid to the anus with nodular contours. The proximal bowel contains stool.

Case 249, Fig. 2B. Lateral view of the rectum during the same barium enema examination confirms marked narrowing of the bowel. The maximum narrowing occurs 5 cm above the anal verge where the maximal narrowing was noted clinically. The mucosa here seems frayed. The contours are nodular. Again separation of the bowel by approximately 3.5 cm from the sacral hollow is noted.



evidence of surrounding mass. Repeat barium enema one month later (Fig. 2A and B) revealed increased rectal narrowing with nodular contours and perirectal mass. The differential diagnosis both clinically and radiographically lay between recurrent carcinoma and radiation fibrosis.

The patient was re-admitted to the hospital. Surgical exploration of the abdomen revealed widespread carcinomatosis involving all peritoneal surfaces, omentum and a frozen pelvis. A diverting colostomy was performed. The patient expired one month later and ten months after completion of treatment. Post mortem examination confirmed the presence of widespread metastatic squamous carcinoma.

*Case Report:* RECURRENT AND METASTATIC CARCINOMA OF THE CERVIX FOLLOWING RADIATION THERAPY.

#### DISCUSSION

See discussion following Case No. 250.

#### CASE NO. 250

A 31 year old female was admitted to the hospital because of severe vaginal bleeding and borderline shock. She stated that irregular menstrual periods and intermenstrual bleeding had occurred during the previous 18 months. Bleeding became heavy and almost continuous in the few days prior to admission.

General physical examination was normal. Pelvic examination revealed a large tumor mass with deep central ulceration which replaced the entire cervix and extended into the left fornix. The left parametrium was infiltrated, but the extension did not reach the pelvic wall; the right parametrium was free of infiltration. Brisk bleeding was observed from the central ulcerated area which was controlled with difficulty utilizing electrocautery. The clinical impression was carcinoma of the cervix, Stage II. A biopsy of the lesion was reported as epidermoid carcinoma.

Preliminary radiographic studies included chest x-ray, intravenous pyelogram and barium enema (Fig. 1), and these were normal. The patient was referred for radiotherapy. Treatment consisted of 250 Kv external beam therapy to six pelvic portals along with 9,000 mg-hrs of radium therapy utilizing vaginal colpostat and intrauterine tandem. The course of radiation represented the standard treatment employed at this institution; it lasted for seven weeks, about one week shorter than the average case.

One week following the completion of treatment, pelvic examination revealed no evidence of tumor in the cervix. An active radiation reaction was apparent. The patient complained of frequent loose watery stools. One month after treatment the cervix had healed and only slight thickening in the medial portions of both parametria was reported but no mass. No abnormal symptoms were present referable to bowel or bladder function. There were similar findings seven months after treatment. Nine months after treatment the patient reported low abdominal cramps, tenesmus, frequent urge to defecate but failure to pass stool freely, and the passage of bloody mucus per rectum.





Case 250, Fig. 1. Lateral view of the rectum during barium enema examination shows normal distensibility of the rectum and rectosigmoid. The bowel has a normal relationship to the sacral hollow. The margins are smooth. No masses are noted.

Examination revealed a tender hard mass surrounding the rectum continuous with a mass in the left parametrium which reached the pelvic wall. There was rubbery induration in the right parametrium. The perirectal mass felt sub-mucosal in location with overlying mucosa intact.

Barium enema revealed narrowing of the rectum with thickened folds, nodular contours and perirectal mass (Fig. 2A and B). The impression both clinically and radiographically was that of recurrent advancing neoplasm and the patient was referred to another institution for possible radical pelvic surgery. Sigmoidoscopic biopsies were negative for tumor. At laparotomy, a thorough exploration revealed fibrosis and thickening of the pelvic tissues and sigmoid colon consistent with radiation effects. Multiple biopsies were taken but no tumor was found. A diverting proximal sigmoid colostomy was performed. The patient recovered from surgery without incident and was discharged from the hospital. The time was now approximately one year after the original course of radiation therapy.



A



B

Case 250, Fig. 2A. Posteroanterior view during barium enema examination shows nodular contours and thickened folds in the distal sigmoid, rectosigmoid, and rectum. Arrows point to a small amount of residual opaque material in the bladder from intravenous pyelogram. Nodular indentations of the rectal contours are noted maximal on the left aspect.

Case 250, Fig. 2B. Lateral view of the rectum during the same barium enema examination shows marked limitation of distensibility of the rectum, nodular contours throughout and thickened fold pattern. There is mass around the rectum with the bowel separated from the sacral hollow by a distance of 3.5 cm.

Three weeks later there was a sudden onset of profuse bleeding from the rectum and the mucus fistula of the defunctionalized bowel. The patient was brought to the hospital where she expired despite vigorous transfusion therapy. Post mortem examination revealed mucosal ulceration and thickening of the bowel in the rectum and rectosigmoid. There was blood in the bowel but a specific bleeding source was not identified. Careful gross and histologic examination revealed severe radiation changes but no evidence of carcinoma.

*Case Report:* SEVERE RADIATION CHANGES FOLLOWING THERAPY FOR CARCINOMA OF THE CERVIX.

#### DISCUSSION

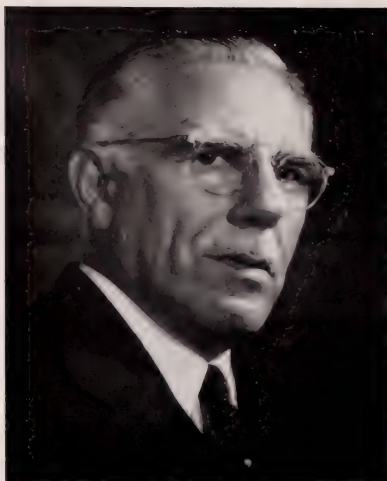
Cases No. 249 and No. 250 are similar in many respects. From the clinical viewpoint they both represent examples of advanced Stage II carcinomata of the cervix with an initial favorable response to a vigorous course of radiation therapy. Complications developed in each case in the latter half of the first year after treatment, and the differential diagnosis lay between recurrent neoplasm and severe radiation effects. Comparison of the barium enema studies in the two cases is particularly challenging. The radiographic features of narrowing of the rectum with limited distensibility, nodularity of contour, and perirectal mass are virtually identical in the two cases. The appearance of the rectum before and after treatment in Case No. 250 makes a striking comparison; the rapid progression of the mass with nodularity in Case No. 249 is equally striking.

When faced with the clinical problem it is important not to leap to false conclusions. Additional radiation therapy to the pelvis in a case of severe radiation reaction with a mistaken diagnosis of recurrent neoplasm leads to disaster. Procrastination in a case of recurrent neoplasm may forfeit the opportunity for additional palliative radiotherapy or radical surgery. Exploratory laparotomy with multiple biopsies, as in both cases presented, must often be advised in order to establish a definitive diagnosis.

#### ACKNOWLEDGMENT

The editors wish to thank Dr. A. Z. Freudenheim, Good Samaritan Hospital, Suffern, N. Y., for permission to publish Cases No. 249 and No. 250.

## THE RALPH COLP AWARDS



DR. RALPH COLP

The Ralph Colp Fund is pleased to announce the granting of two Ralph Colp Awards for 1963. Under the terms of the Fund, established by colleagues, friends, and patients of Dr. Ralph Colp, distinguished American surgeon, illustrious past President of the Medical Board of The Mount Sinai Hospital, teacher and innovator in gastric, biliary and intestinal surgery, a prize is given for the best paper published in the Journal of The Mount Sinai Hospital by a member of the House Staff or by a junior member of the Attending Staff. Preference is given to a paper dealing with a surgical subject but is not so restricted.

The Prize Committee, consisting of the Chairman of the Medical Board Committee on Medical Education, Dr. M. Ralph Kaufman, the Director of Surgery, Dr. Allan E. Kark, and the Editor of the Journal of The Mount Sinai Hospital, Dr. Lester R. Tuchman, unanimously recommended an award to Dr. Demetrius Pertsemilidis, Resident in Surgery, for his paper entitled, "Neonatal Gastric Perforation," and to Dr. Eugene Ainbender, Research Associate in Pediatrics, as co-author of a paper entitled, "A Substance Obtained from a Staphylococcus Which Rapidly Enhances Resistance to Infection."

Each paper was considered of such merit that the Committee was impelled to grant two awards. The co-authors with Dr. Ainbender are not eligible to share the award under the terms of the grant.

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DR. H. LILIENTHAL, 1861-1916



## FOREWORD

On July 1, 1962, the Division of Cardio-thoracic Surgery of The Mount Sinai Hospital was formally established. Yet the inception and practice of thoracic surgery at the hospital preceded this event by almost fifty years. Indeed, history records that one of Mount Sinai's sons, Howard Lilienthal, played a major and pioneer role in the evolution of thoracic surgery as a specialty. Doctor Lilienthal's contributions to the field ranged far and wide and included surgical approaches to pulmonary tuberculosis, cancer of the lung, suppurative lung diseases and empyema—to mention just a few. A significant landmark in the surgery of esophageal cancer was Lilienthal's report of the posterior mediastinotomy approach which he read before the American Surgical Association in June of 1921. Although four subsequent patients succumbed, his initial patient survived for more than one year dying from the effects of recurrent cancer and a tracheo-esophageal fistula. Lilienthal's crisp epicritical note, published with details of the necropsy findings (*Ann. Surg.*, Sept. 1922) is as sound today as when it was written: "This case and others in which the first stage only was performed have taught me: First, that the operation as planned is feasible. Second, that in order to succeed the patient must present himself while the disease is still localized within the oesophagus. Third, that much wider resection of the oesophagus should be made than would appear necessary judging by the extent of the visible lesion." The years which have followed have seen The Mount Sinai Hospital rise to unquestioned pre-eminence in the surgery of esophageal cancer. John Garlock, writing in the 1941 Lilienthal *Festschrift* asserted: "We have now reached a stage in the surgical therapy of cancer of the esophagus where we can assure the patient of a reasonable chance of survival. Dr. Lilienthal's contribution to the subject indicated the feasibility of safe surgical attack and gave encouragement to surgeons in later years to extend the field of applicability of the principles so clearly enunciated by him."

Lilienthal's detailed account of lobectomy in the treatment of bronchiectasis, published in 1922, represented the first extensive clinical report in the medical literature on the subject. His colleague, Evarts A. Graham noted: "Although his mortality figures in those early cases would have been discouraging to many, they did not shake his resolute and indomitable pioneering spirit. Moreover, his series was a demonstration that it is not merely a chance lucky patient who will survive the operation but that in good risk cases the patients are likely to survive. These operations of his at that time without the benefit of modern diagnostic methods of accurate localization and without many of the modern technical procedures which increase the safety of the operation, and even in the face of hostile criticism from some of his colleagues, are good evidence of his courage, his self-reliance and his independence."

Lilienthal's classic work, *Thoracic Surgery*, in two volumes, published

in 1925, was the first American treatise on the subject. Here one sees the author's broad experience meticulously set down. From the first chapter on "General Considerations" until the last, "Military Surgery," these six hundred and ninety-four pages display basic approaches to thoracic disease which are as applicable today as they were then.

In an attempt to give proper honor to the memory of Howard Lilienthal, a series of memorial lectures has been instituted here at the hospital. The speakers for the academic year 1963-1964 are listed below:

- |                 |   |
|-----------------|---|
| Sept. 7, 1963.  | Myron W. Wheat, Jr., M.D., Associate Professor of Surgery,<br>University of Florida College of Medicine.<br>"Ultrastructure Autoradiograph and Lysosome Studies in<br>Myocardium."  |
| Sept. 21, 1963. | James A. Helmsworth, M.D., Associate Professor of Surgery,<br>University of Cincinnati College of Medicine.<br>"Considerations in the Surgical Treatment of Transposition<br>of the Great Vessels."   |
| Nov. 16, 1963.  | Frank Gollan, M.D., Chief, Radio-Isotope Service, Veterans<br>Administration Hospital, Coral Gables, Florida, and Assistant<br>Professor of Medicine, University of Miami School of Medicine.<br>"Carbon Dioxide, Waste Product or Elixir?"   |
| Dec. 14, 1963.  | Philip Samet, M.D., Chief, Cardiopulmonary Laboratory, Mount<br>Sinai Hospital, Miami Beach, Florida, and Associate Professor<br>of Medicine and Physiology, University of Miami School of<br>Medicine, and William H. Bernstein, M.D.<br>"Hemodynamic Considerations in Complete Heart Block." |
| Jan. 18, 1964.  | Willem J. Kolff, M.D., Chief, Department of Artificial Organs,<br>Cleveland Clinic.<br>"To Live Without Heart and Kidneys."   |
| Feb. 29, 1964.  | Eugene Braunwald, M.D., Chief, Cardiology Branch National<br>Heart Institute.<br>"The Mechanism of Action of Digitalis."  |
| March 21, 1964. | Pierre M. Galletti, M.D., Associate Professor of Physiology,<br>Emory University School of Medicine.<br>"Physiological Basis for Assisted Circulation."   |
| April 18, 1964. | Dwight E. Harken, M.D., Clinical Professor of Surgery, Harvard<br>Medical School.<br>"I. A New Caged-Ball Aortic and Mitral Valve and II. Moni-<br>toring and Controlled Respiration in Critically Ill Pa-<br>tients."  |

The following series of papers are respectfully published in memory of Howard Lilienthal and his many contributions to the field of thoracic surgery.

Robert S. Litwak, M.D.

# **I. A New Caged-ball Aortic and Mitral Valve and II. Monitoring and Controlled Respiration in Critically Ill Patients\***

DWIGHT E. HARKEN, M.D.†

The Howard Lilienthal Lecture Series honors a surgeon pre-eminent among the illustrious of this hospital and of the world. We who participate in this homage are grateful for the borrowed distinction.

All thoracic surgeons of my generation have been directly influenced by Dr. Lilienthal. The courageous tenacity with which Dr. Lilienthal persisted in his bold but disappointing adventures with lobectomy and pneumonectomy had more relevance to the continuing efforts, in the early dark days of mitral valve surgery, than most of you know nor do I care to recall.

This salute to Dr. Lilienthal will be in two parts. The first will describe a new caged-ball valve for aortic, mitral or combined replacement. The second part will point up monitoring and respiratory support techniques that have made this complicated surgery safer. The broader significance of these monitoring methods and the cardio-respiratory support, to other critically ill patients, is apparent.

## **I. A NEW CAGED-BALL AORTIC AND MITRAL VALVE**

The new valve is more versatile than its predecessor which was the first caged-ball valve to be used successfully in humans at the anatomic site. The new valve (Fig. 1) has a number of advantages:

- a) Three sizes cover the spectrum for adult humans for aortic, mitral and combined replacement. In this versatility they are unique.
- b) The cages are of titanium, which is lighter, stronger and less reactive than other metals.
- c) The ball and valve orific ratios are such that low activation forces are required on a relatively large ball. This reduces the opening and closing time lag.
- d) Time lag in opening and closing the valve or "activation" factors inherent in overcoming the inertia of solid (though isobaric) silicone balls is further reduced by making them hollow.

Lecture presented April 18, 1964, at The Mount Sinai Hospital, New York, N. Y.

\* From the Department of Surgery, Peter Bent Brigham Hospital, Boston, Mass., and Surgical Research Laboratory, Harvard Medical School, Boston, Mass. Supported in part by United States Public Health Grants HTS-5231, HE-02419 and HE-08698, and a very special acknowledgement of appreciation to Nathan Reifler, his family and friends.

† Clinical Professor of Surgery, Harvard Medical School; Surgeon and Chief of Department of Thoracic Surgery, Peter Bent Brigham and Mount Auburn Hospitals.

e) A unique off-set interface between ball and base eliminates the danger of sticking and broad ball and cage top contact reduces wear.

f) Tapered, elliptical struts offer minimal resistance to forward blood flow and hold the walls of the aorta and ventricle away from the ball raceway to prevent interference with ball action.



FIG. 1. Three sizes of caged-ball valves cover the aortic and mitral, human adult size spectrum. The two white balls (hollow) are contrasted with the obsolete solid balls. Note smooth outflow tract. The only metal exposed is in high velocity axis to prevent pannus extension. Strut ends at open apex present recessed tips for atraumatic leading surface to septum and ventricle. Ball side of strut apex provides broad contact surface to reduce wear.

g) The skirt is of four-ply Dacron<sup>®</sup>\* of coarse porosity and swedged to the titanium base so that no flat atrial metal surface is exposed to propagate thrombus and emboli. Coarse skirt texture favors fibroblast infiltration.

h) The hollow silicone ball reduces opening and closing impact that may produce disturbing sounds and sensations.

i) The open valve top eliminates streamer thrombus problems from apical crossed struts.

\*© A DuPont product

j) Points at the open valve apex are recessed presenting a smooth distal leading strut surface.

k) The ball is glazed to reduce blood reaction. In the wet state this minimizes surface cohesion between ball and cage.

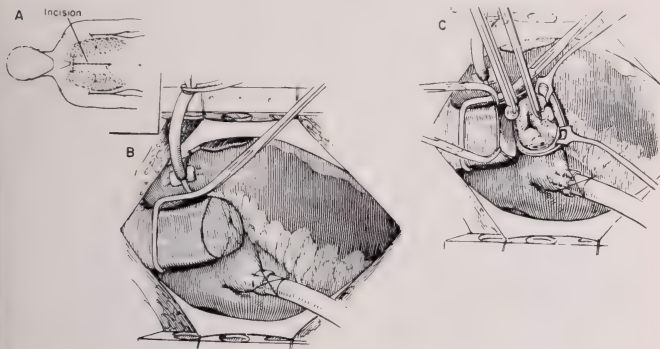


FIG. 2. A) Low "T" incision with sternal division deviating to right in aortic area and left over ventricles. B) Isolation of vessels for occlusion. Combined caval cannulation not generally used. Prefer right atrial and femoral take-off allowing retrograde coronary perfusion. C) Low transverse aortotomy with immediate cold coronary perfusion.

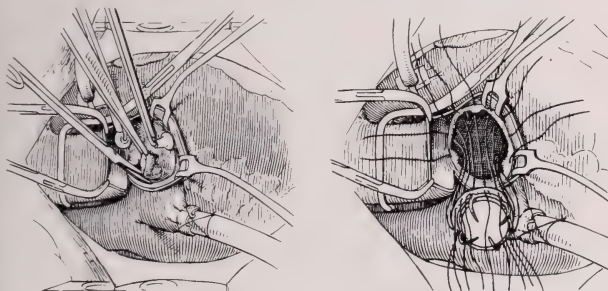


FIG. 3. Cold coronary perfusion during meticulous valve excision.

FIG. 4. Multiple sutures (approximately 30) maintained in orderly pattern by 9" ring (not shown).

### *Aortic Valve Replacement*

Illustrations 2A, 2B and 2C demonstrate exposure of the aortic base through a sternal splitting incision, transverse aortotomy and cold coronary artery perfusion. The right atrium and femoral vein are cannulated for venous

take-off. The damaged aortic valve is excised and great care is exercised to avoid losing calcium particles that may later become emboli. The calcium is removed as completely as possible. Occasionally this involves perforation of the aortic wall, the interventricular septum or major mitral leaflet. Such defects are carefully repaired if, as and when they occur. The chamber of the left ventricle is packed to avoid loss of calcific particles and fine suction filters are placed in the coronary return sucker lines.

After excision of the valve and meticulous calcium removal, the skirt is fixed with multiple (generally 30) Tefdac®\* (000) sutures. These may be kept in order by affixing to a stainless steel spring ring 9" in diameter (Figs. 3, 4). After all sutures are placed, the valve slides into place and the sutures are tied for firm fixation (Fig. 5). The valve in place, the aorta is closed

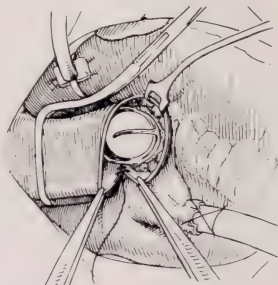
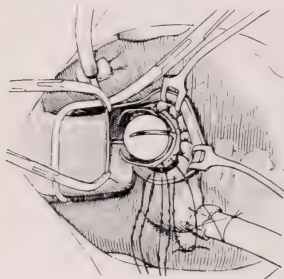


FIG. 5. The valve is fixed firmly in subcoronary position by overlapping sutures.  
FIG. 6. Transverse aortotomy closure.

with Tefdac® (0000) running sutures (Fig. 6). As the aortotomy is closed (Fig. 7) air in the left atrium, left ventricle and aorta is flushed out with  $\text{CO}_2$ . Occasionally this maneuver is associated with left to right shunt through an open foramen ovale and venous return is interrupted. This quickly rectifies itself if the  $\text{CO}_2$  flow is stopped. The obstructing tourniquet on the pulmonary artery is released every ten minutes during by-pass to allow perfusion of the cooled lungs ( $28^\circ\text{C}$ ). The lungs are slowly ventilated with 80%  $\text{O}_2$  during by-pass (Fig. 8).

With the opening of the aorta the left ventricle is decompressed through a coronary sucker in the apex of the left ventricle.

A warning must be sounded regarding the over use of calcium, isoproterenol, norepinephrine or other ionotropic drugs at this phase in patients who have massive concentric left ventricular hypertrophy. Such tetany of the ventricle may occur as to cause lethal outflow tract obstruction or reduced stroke

\*R J. A. Deknatel and Son, Inc., Queens Village, L. I., N. Y.



volume. This "iatrogenic obstruction to cardiac output" will be the subject of a subsequent report.

For six hours after operation (longer if indicated), the endotracheal tube is retained for controlled respiration as will be described later.

Thirty-six hours after operation, if there is no contraindication the patient is placed on heparin. This heparinization is tapered off just before discharge from the hospital. We do not believe that prolonged Coumadin®\* "anti-coagulation" confers any added protection against embolization when using this new type of valve.

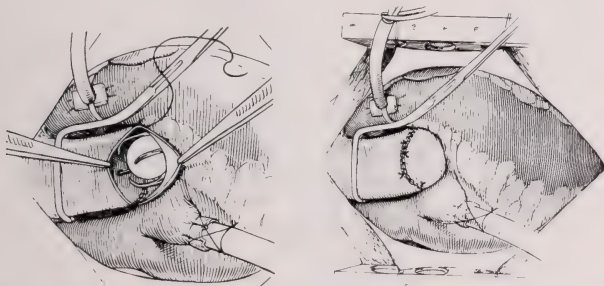


FIG. 7. The aortotomy incision is closed at the ends first where exposure is more difficult. The anterior portion is closed after air has been washed out with carbon dioxide.

FIG. 8. The aortotomy closure is complete, the clamp is removed and the ventricle decompressed through apical suction.

### *Mitral Valve Replacement*

The late experience with mitral valve annuloplasty, even in non-calcific valves was disappointing, even though competence was restored and the upward herniation of the incompetent mitral complex was corrected. The inexorable march of the valve disease process and the abnormal leaflet coaptation trauma has brought most of these people back for further operations (Fig. 9).

The next step in the evolution of surgery for mitral insufficiency was that of partial replacement in which the mural leaflet was extended by an Ivalon®† "sandwich baffle" to correct the space deficiency (Fig. 10). This carried a better and more lasting hemodynamic result, but unfortunately many major aortic cusps of the mitral complex were damaged by repeated systolic trauma against the Ivalon® buttress. The substitution of the caged-ball valves for compromise procedures has been widely adopted. Hopefully the valve described here has advantages over the mitral Starr valve. Refer-

\*® Endo Laboratories, Inc., Richmond Hill, N. Y.

†® Clay Adams Inc., Chicago, Ill.

ence has already been made to some of these. The base is less bulky, there is less metal exposed for pannus formation and therefore less likelihood of embolization. The open cage top reduces the danger of streamer thrombi and embolization. The skirt of coarse porous four-ply Dacron® is not only smaller but more flexible and more readily infiltrated with fibroblasts.

The mitral valve was originally placed through a right-sided or sternal splitting incision. This was awkward and a left-sided approach was developed.

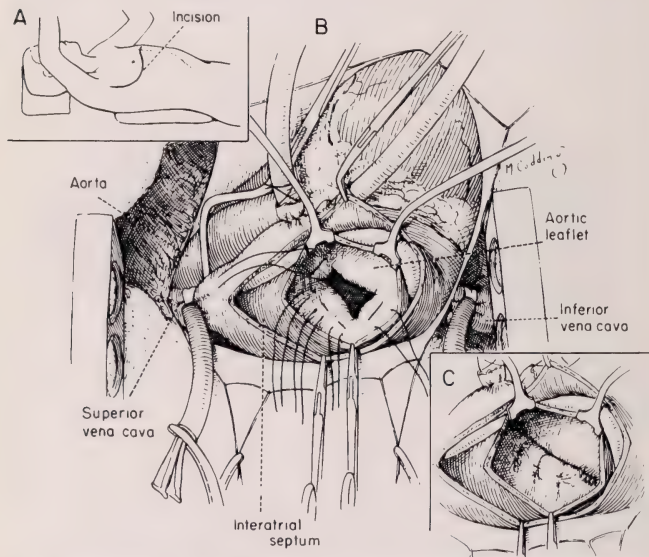


Fig. 9. Now obsolete, the "annuloplasty" was performed through right atrium and septum. Sutures buttressed by plastic sponge held, but leaflets contracted and insufficiency often recurred.

The main objection of the left approach was that of venous cannulation for by-pass. An especially contoured, tapered cannula\* renders right ventricular cannulation simple (Fig. 11). The right ventricle is exposed by appropriate marsupialization of the pericardium and 30° backward rotation from the direct left lateral decubitus position (Figs. 12A, 12B). Having cannulated the right ventricle, the patient is then rotated 30° forward, beyond the direct left lateral position to expose the left atrium (Fig. 12C). Mitral valve excision and replacement are then straightforward (Figs. 13, 14).

\* Manufactured by the Davol Rubber Company, Providence, Rhode Island.

When the mitral valve is being removed and replaced the aortic valve may be competent enough to allow continuous perfusion of the coronaries. If reflux interferes with vision, the tourniquet about the upper part of the ascending aorta can be used to occlude the aorta intermittently (Fig. 12B). The chordae tendinae and apices of the papillary muscles are removed with the valve complex. However to place the prosthetic valve base away from the

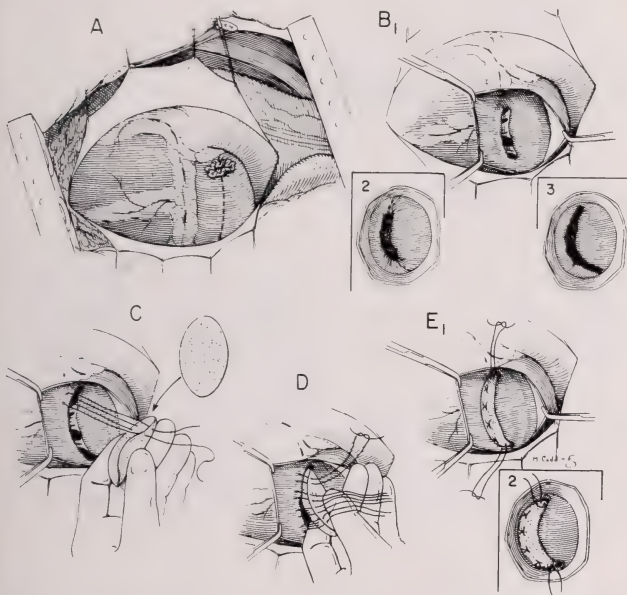


FIG. 10. The "sandwich baffle" was placed via left thoracotomy as in Figure 12. Major leaflet often contracted as in "annuloplasty" with recurrence of regurgitation.

aortic valve and left ventricular outflow tract a one to two centimeter cuff of the major mitral leaflet is not excised (Fig. 13B).

When the combined valve replacement is necessary, the exposure of the base of the aorta is expedited by incising the residual margin of the mitral major leaflet (Fig. 15A). Exposure is facilitated by traction sutures (Fig. 15B) for excision of the aortic valve. Having excised the aortic valve, traction sutures in the aortic commissures secure aortic base exposure and often allow coronary perfusion. As soon as the prosthetic aortic valve is placed, coronary

perfusion is possible by releasing the tourniquet on the aorta. Coronary perfusion as well as intermittent perfusion and ventilation of the lung are important.

Experience now with approximately 300 valve replacements makes it possible to say that favorably selected aortic and mitral valve prostheses can be inserted with a mortality of less than 10%. Those in failure or who have had previous surgery have a considerably higher risk. Combined valve replacement of more favorable cases (not in failure) without previous sur-



FIG. 11. The firm tapered tip facilitates cannulation of the right ventricle or atrium.

gery should be operated with a mortality of approximately 20%. We should and will improve.\*

#### II. MONITORING METHODS AND CONTROLLED RESPIRATION IN CRITICALLY ILL PATIENTS

The second portion of this commemorative discussion attempts a broader purpose. It reflects briefly on some monitoring methods that have become standard practice in the postoperative care of patients after more complicated open heart surgery. Finally the technique of prolonged controlled respiration has evolved from open heart surgery. These methods should be ap-

\* This portion of the presentation was illustrated by motion picture films of the techniques involved.

plied to other patients such as those critically ill after massive gastrointestinal bleeding, barbiturate poisoning or massive myocardial infarction.

Monitoring simply facilitates and extends observation. The more we do for our patients the greater our obligation for observation. The more precise our observations, the better regulated and appropriate are those things we do. Individually the techniques of monitoring outlined here, even the therapeutic

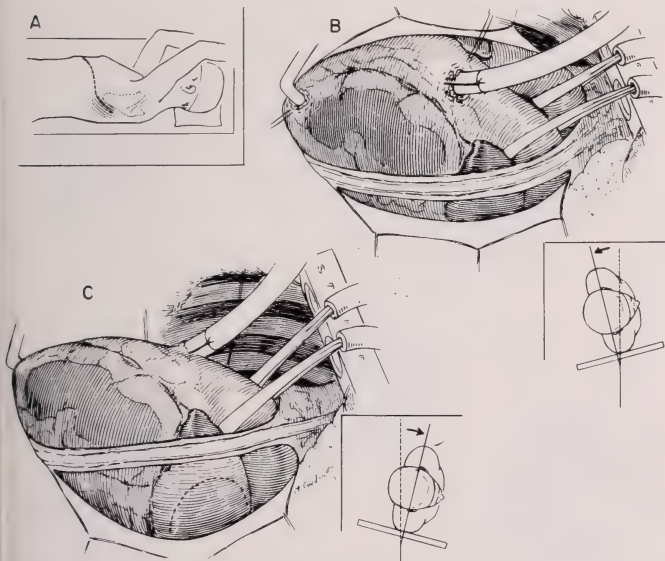


FIG. 12. A) Lateral decubitus with full 4th interspace incision and division of costal symphysis. B) The pulmonary artery and aorta are isolated. The patient is rotated back to expose the right ventricle for cannulation after the heart is lifted and rotated by pericardial marsupialization. C) Reverse rotation for left atrial exposure.

implications of those measurements, are for the most part well known. However the entire interrelated constellation has not been routinely used in the intensive care of critically ill patients.

Valve replacement is only one facet of the surgical program requiring more complex observation and care. Your own Doctors Litwak and Gadbois have extended the need by discovering that the mere use of multiple human transfusions and homologous transfusion reactions may have caused many of the difficulties of our early open heart procedures. They have added safety through hemodilution and reduced the number of donors necessary for an operation.

We use approximately equal parts of blood and Ringer's solution, adjusting the pH to 7.4 and adding glucose. This technique of hemodilution for by-pass has improved the microcirculation and moderate hypothermia has further reduced the metabolic deficits of perfusion. Some metabolic acidosis can be corrected with organic buffers (Triss<sup>®</sup>\*) in the postoperative period.

As procedures become more complicated, variables are added and more complex monitoring is required. There is no defense for such reckless state-

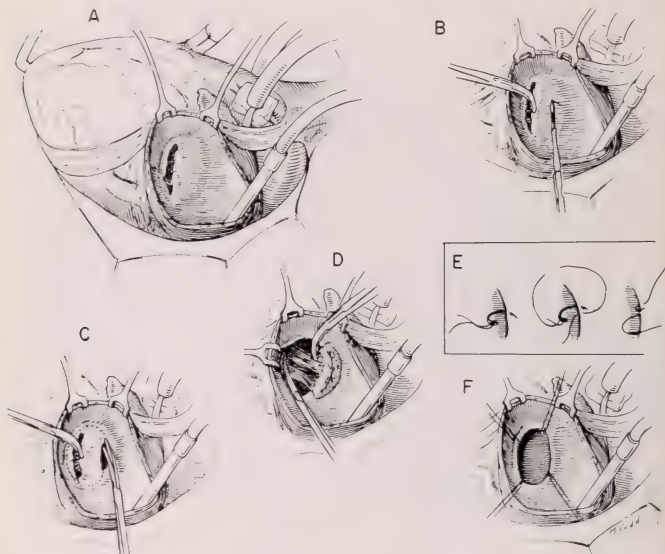


FIG. 13. Valve exposure. B) A generous curtain of major mitral leaflet places the prosthesis away from the aortic base and outflow tract. C-D) The valve complex is excised to the tips of the papillary muscles. F) Exposure is facilitated by traction sutures.

ments as "we've given up all monitoring during by-pass except flow." A surgeon who carries such thinking to the postoperative phase should agree to operate only patients who can present no possible controllable deviation from the physiologic norm. This denies care to the neediest and can hardly expect to improve existing therapy.

The recurring question is, what to monitor and what to do about the information obtained? Simplicity and ready availability are relative terms. We will, hopefully, soon have readily available, other important data related to

\*<sup>®</sup> Abbott Laboratories, North Chicago, Ill.



serial cardiac outputs, intra- and extravascular fluid estimation and so forth. It is important to emphasize here what is available now and routine.

Through common usage the following techniques have become standard to the point of simplicity.

Prior to surgery a polyethylene catheter (P.E. 90-160) is passed into the ulnar or brachial artery to or near the aortic arch. Central arterial pressures can then be followed. This can be, but need not be, recorded by elaborate

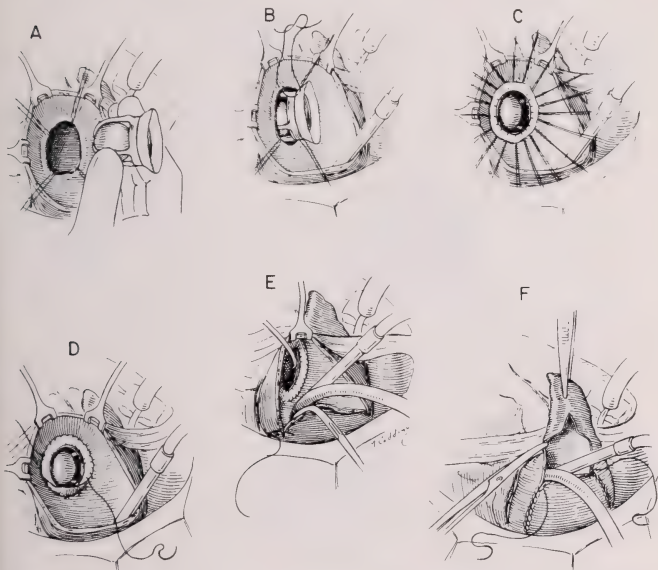


FIG. 14. The mitral prosthesis is secured by central interrupted sutures and the knots are covered by the skirt sutured to the atrium by running sutures.

devices. An accurate, constant mean pressure reading can be followed by means of a mercury manometer attached to the bedside table. The limitations of conventional blood pressure cuffs are soon evident. Often peripheral vascular constriction leads one, using an arm cuff, to conclude that the pressure is low when the use of such drugs as norepinephrine may have produced a very high central pressure. The disadvantages of such misinformation to renal and other peripheral perfusion as well as in myocardial pressure work are evident. This cannula is a ready source of arterial blood sampling. These cannulae generally remain in situ for 24 to 48 hours.

Similarly and through the same cutaneous cut-down site the anticubital

vein can be cannulated with a polyethylene catheter (P.E. 250) passed to the superior cava. This allows easy determination of central venous pressure. It again is a ready source for venous blood sampling and a port for delivering such solutions as vasopressors, glucose, potassium, Mannitol and organic buffers that might cause vein and tissue damage if delivered into smaller peripheral veins. Left atrial and pulmonary artery pressure monitoring have

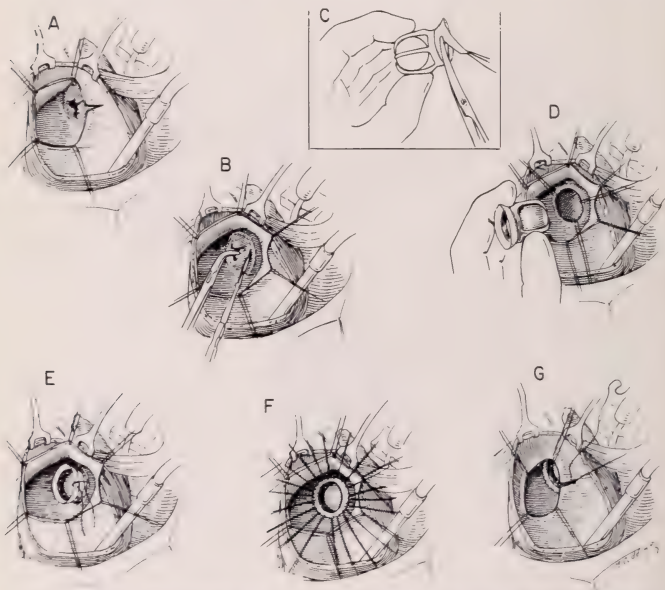


FIG. 15 Aortic valve excision and replacement can be carried out after the mitral valve has been excised. C) The skirt can be reduced in size if necessary. D) Exposure of the aortic base is facilitated by traction sutures in the commissures.

relevance to heart surgery rather than to the general care of other critically ill patients.

The electroencephalogram may have been overused. Remarkable changes of uncertain significance may occur throughout anesthesia and surgery with and without by-pass. Following surgery the level of the patients' consciousness provides as satisfactory an estimate of brain perfusion as does the electroencephalogram. This device then becomes an instrument for specific rather than general use.

The common use and advantages of a cardioscope in monitoring, with fre-

quent added complete electrocardiographic survey, are such as to require little more than passing reference. So much can be done to control heart rate, myocardial ischemia and the arrhythmias that this continuous passing pattern on the screen is invaluable. It is also a wholesome reminder to the surgeon and the anesthesiologist that they have an obligation to understand the electrocardiogram.

The indwelling catheter providing minute to minute or hourly urinary output contributes guides to the rational use of blood, fluids, diuretics, glucose and potassium.

The frequent assay of such factors as electrolytes, blood chemistry and the hematocrit need no comment except to condemn the common correlation of sampling with the cycle of the working day. There is too little of this form of monitoring and particular neglect of the potassium/digitalis relationship.

It is widely assumed that the patients' body temperature as recorded by the nurse on the chart has high correlation with fact. The routine use of a rectal thermocouple affords a whole new dimension of accuracy and convenience.

Probably the greatest recent contributions to the care of the critically ill have been in the areas of acidosis and alkalosis (metabolic and respiratory). The patient on cardio-pulmonary by-pass and his postoperative care have forced an incredible crash program to improve technical methodology and physician understanding. It is now easy to obtain accurate arterial and venous  $pO_2$ , pH and  $pCO_2$  values frequently and easily. Furthermore appropriate therapy can often be direct and immediate. The most effective and most recent extension of therapy here is long-term assisted or controlled respiration.

Over the years there has been reluctance to leave a cuffed endotracheal tube in place for prolonged periods (one or more days). This apprehension may stem from early oversized rubber tubes that caused larynx or cord damage. Now, however, this has lost some of its validity as the non-reactive plastics can, with a smaller outside diameter, provide greater lumen. Such endotracheal tubes have allowed postponement of or avoidance of tracheostomy; this is important for tracheostomy, at times invaluable, but does carry a high incidence of associated pulmonary infection. In addition whether transoral or transtracheostomy, the cuffed endotracheal tube provides opportunity for meticulous tracheobronchial toilet. Finally this intubation makes possible the use of one of our most valuable therapeutic aids, mechanical assisted and controlled respiration. When a demand valve allows mechanical augmentation of the patients' respiratory effort, this is assisted respiration. When the patients' inspiratory forces are taken over by the mechanical ventilator, it is called controlled respiration. This "control" may come about through a variety of mechanisms including patient exhaustion, paralysis of respiratory muscles or commonly, in this situation, by elective wash-out of  $CO_2$  to the point of respiratory center paralysis.

There are many advantages of controlled respiration. A few of these advantages and some technical notes include:

a) The work of respiration is taken over by the machine. This conserves the patients' energy sources and reduces metabolic deficits.

b) Postoperative discomfort can be more aggressively combatted with analgesics for there is little concern over respiratory depression.

c) Associated ventilation with  $N_2O$   $O_2$  mixtures allow controlled analgesia and amnesia. The patients' conscious level can be titrated with  $N_2O$ . Oxygen levels of more than 75% in the ventilating mixtures are to be avoided. Careful humidification is important. Reaction to or inability to tolerate the endotracheal tube may require Thorazine®\* or Phenergan®†.

d) The endotracheal tube allows improved tracheobronchial toilet and reduces pulmonary complications.

e) Mechanical inflation of all areas of both lungs reduces intrapulmonary vascular shunts and greatly improves oxygenation.

f) Mechanical ventilation may compensate for metabolic acidosis by inducing respiratory alkalosis.

g) Loss of pulmonary compliance is prevented by frequent alteration in intrapulmonary pressures (intermittent "sighing" = q 15").

h) After thoracotomy, bleeding tendencies are less because anoxia and vascular spasm are corrected and there is the hemostatic force of local mechanical compression by the inflated lungs on denuded surfaces.

It is not only impossible but beyond the province of this presentation to attempt credit to the host of workers including Engstrom, Björk, Byrd, Craaford, Dammin, Burnap, Bennett and Emerson who have pioneered methods and machines useful to these purposes of controlled respiration. This is an area of cardinal importance that has been given vital urgency by heart surgery. It must now have wider application in other critically ill patients. The standard mechanical devices widely available are mostly sufficient when used effectively. It matters less what is used than how it is used.‡

In closing we again salute Dr. Lilienthal and you of this great hospital who so ably continue his tradition.

It gives me pleasure to think that Dr. Lilienthal would have been intrigued by new and versatile mechanical heart valves; that he would have urged better methods in the care of the patients who need valves, that he would have delighted in the evident inter-disciplinary synergism of these new therapies; finally, that he would have applied these useful techniques to other critically ill patients.

\*® Smith, Kline & French Laboratories, Philadelphia, Penn.

†® Wyeth Laboratories, Philadelphia, Penn.

‡ These techniques were demonstrated by motion pictures.

# Ultrastructure Autoradiography and Lysosome Studies in Myocardium

MYRON W. WHEAT, JR., M.D.\*

I suspect it seems strange to most of you to find yourselves listening to a surgeon talk about the ultrastructure of anything. However, in the overall perspective this need not seem so strange. In recent years, strides have been made in all fields of medicine, particularly in the areas of surgery and surgical research. Nowadays, it is well accepted for surgeons to be engaged in research using flow meters, computers and numerous other electronic devices. The electron microscope is merely another electronic tool. Since the surgeon is basically an anatomist, it follows that we may study anatomy at the most basic morphological level presently available, the ultrastructural level. My discussion will fall into two parts. The first part has to do with the localization of digitalis in heart muscle using ultrastructure autoradiography. The second part concerns the lysosomes in human myocardium.

## PART I. LOCALIZATION OF DIGITALIS IN MYOCARDIUM BY ULTRASTRUCTURE AUTORADIOGRAPHY

A problem that has interested me has been the localization of digitalis in heart muscle. Several years were spent attempting to localize digitalis in heart muscle by chemical methods (1). This attempt was fruitless presumably because the amount of digitalis actually present in the myocardium at any one time is so small as to make it difficult to detect. More recently Caro (2, 3) has made possible the application of the techniques of autoradiography to the subcellular level with a resolution of the order of 0.1 micron. As a result of these advances, and with the availability of tritiated digoxin, we decided to see if these techniques could be combined to shed light on the distribution of digitalis in the heart muscle of dogs.

During the past year, Dr. Eugene Tubbs, working with Dr. Lamar Crevasse and me, carried out the following studies. Mongrel dogs were given an intoxicating dose of tritiated digoxin over an 18 hour period and then sacrificed. Left ventricular samples were taken from the beating heart, immediately immersed in buffered osmium tetroxide, minced into approximately 1 mm cubes, and fixed for one hour. The tissue was then dehydrated in alcohol and embedded in Epon 812.

It soon became apparent that the concentrations of alcohol used in the dehydrating process and the period of time that the tissue was left in the alcohol were very important. The exposure of the tissue for five minutes to an alcohol concentration of 80 per cent caused a significant loss of labeled material, i.e., the concentration of labeled material removed was found to be twice as great as that removed when the tissue was left in the alcohol for only 30 seconds.

Lecture presented September 7, 1963, at The Mount Sinai Hospital, New York, N.Y.

\* Section of Thoracic and Cardiovascular Surgery, Department of Surgery, College of Medicine, University of Florida, Gainesville, Florida.

In these experiments, therefore, the muscle was dehydrated in the various strengths of alcohol for only 30 seconds.

Following embedding, the tissue was sectioned, mounted on parlodion-coated nichrome grids, and prepared for ultrastructure autoradiography by coating the tissue with a photographic emulsion. When the photographic emulsion has been applied correctly to the supporting grids the emulsion consists of a monolayer of silver bromide crystals approximately 0.1 micron thick (Fig. 1A). After the completion of the photographic process the silver bromide crystal is no longer present. Instead a filament of silver has developed which begins from the latent image (Fig. 1B). The silver filaments may assume

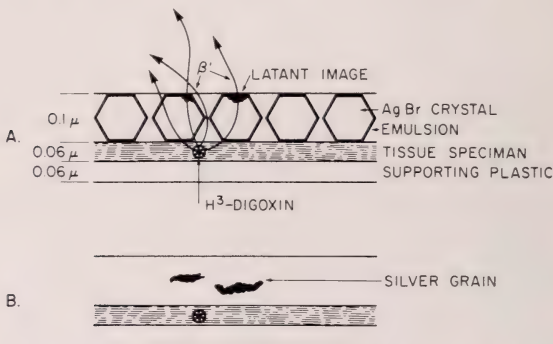


FIG. 1. A. Latent image which is formed by collision of a  $\beta$  particle with a crystal of silver bromide occurs during exposure time. With thin tissue sections and a monolayer of silver bromide overlying tissue, the chance of a point source of radioactivity resulting in latent image production is almost 100%.

FIG. 1. B. After development silver filament which has grown from latent image remains over site of radioactivity (redrawn after Caro).

two basic forms shaped either as a comma or present in a more complicated form, depending upon the type of developing material utilized (Fig. 2). The point source of radioactivity has been determined to be at the head in the comma form, or at the center of the more complicated forms. The radioactivity present in this particular tissue resulted in prime autoradiographs after two months of exposure.

The finished grids were viewed with an RCA EMU 3C electronmicroscope and photographs of the labeled tissue made. These were compared with photographs of control tissue and of grids containing no tissue treated in an identical manner.

One hundred sections of tritiated digitoxin labeled tissue, each comprising a  $100 \times 100$  micra area, had an average number of grain counts of 28. Counts were made in a similar manner using nonlabeled tissue and blank



grids. Both of these controls showed average counts well below that of the tissue labeled digoxin (Table I). It should be emphasized that the technique of autoradiography identifies the radioactive isotope only. This means that each nuclear track is not necessarily a representative of digoxin in that particular site. The amount of label which represents intact drug or its breakdown products cannot be determined by this technique.

The sites of isotope activity were then localized to determine whether they



FIG. 2. An electron micrograph of a portion of a grid incubated with isotope and a layer of photographic emulsion but no tissue. The variously formed nuclear tracks are shown. Original magnification 12,750.

were primarily present over the A band, Z line, H disc, I band, or the mitochondria. The area occupied by the mitochondria and the sarcomeres was estimated by cutting out these structures from ten random photographs and weighing the paper on an analytical balance. All of the remaining portions of these photographs were weighed as another unit. The area occupied by the mitochondria and sarcomeres was then expressed as a percentage of total weight of the photograph (Table II).

The results of this study show that in digitalis intoxicated dogs the nuclear tracks, and presumably the labeled digoxin, are found in the myocardial cell primarily in association with the myofibril (Figs. 3, 4, 5). Of the

total number of nuclear tracks counted, 77 per cent were found to overlie the sarcomeres, 18 per cent to overlie the mitochondria, and 5 per cent to overlie all other structures. The localization of the label over the sarcomere is meaningful in terms of the estimated total per cent area of the sarcomere with respect to other structures. Whereas 55 per cent of the tissue area studied was occupied by sarcomeres, approximately 77 per cent of all nuclear tracks were over sarcomeres. If the distribution of these tracks were on a random basis only, one would expect the number of nuclear tracks over a structure to be proportional to the area occupied by that structure.

A high percentage of the label apparently is localized at the A band of the sarcomere. As is shown in Figure 3, most of our tissue is in the contracted state and therefore the A band is the predominant area in the sarcomere. Localization of the label over the I band would be impossible

TABLE I

H <sup>3</sup> -Digoxin	Control Tissue	Blank Grids
28 (12-56)	3 (0-6)	4 (2-7)

Silver grains present in randomly selected sites 10,000 square micra in area. Upper values represent average number counted in one hundred observations. Lower values represent maximum and minimal values. Exposure time two months.

TABLE II

Sarcomere	Mitochondria	Other
54.6	24.7	20.7

Percentages of total area of random photographs occupied by sarcomeres, mitochondria and other structures. The relative areas were obtained by cutting out the various structures in ten random photographs, weighing them and expressing the various areas as a percentage of the total photograph weight.

to determine with tissue in the contracted state. During the progress of this work, however, Smith and Fozzard of Washington University, St. Louis (4), reported findings similar to ours using ultrastructure autoradiography. Their tissue was fixed in a relaxed or a stretched state with the I bands visible and their labels still appeared to be localized to the A band of the sarcomere.

In summary, our studies of the localization of digitalis in the canine myocardium using ultrastructure autoradiography suggest that digitalis, or at least the radioactive label, is primarily localized to the sarcomere. Of the total number of nuclear tracks counted, 77 per cent were over the sarcomeres, whereas the sarcomeres occupied only 55 per cent of the tissue area.

#### PART II. LYSOSOMES IN HUMAN MYOCARDIUM

Next, I wish to discuss our work concerning another aspect of the myocardial cell, the lysosome. The lysosome is a small intracellular organelle,

originally postulated by deDuve in 1955 on the basis of enzyme studies of rat liver (5, 6). Since that time the lysosome concept has advanced from a biochemical speculation (as originally proposed by deDuve) to the status of a reasonable morphological entity as a result of many correlative studies (7-16). At the present time the lysosome may be described as an intracellular organelle around 1 micron in diameter having a relatively sturdy well-

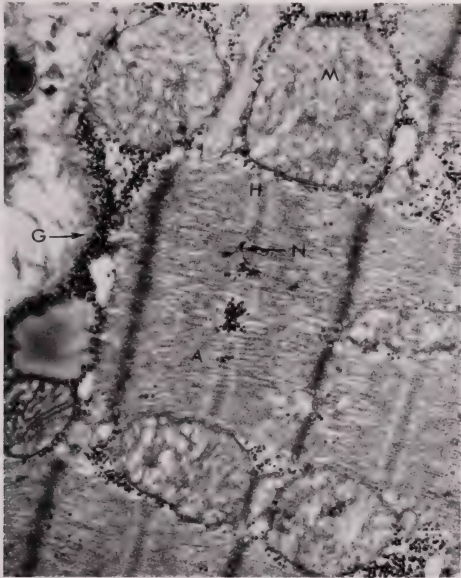


FIG. 3. Ultrastructure autoradiograph of dog myocardium showing two comma shaped nuclear tracks localized in the A band area of a sarcomere. A band = A; H disc = H; Z line = Z; mitochondria = M; glycogen granules = G; nuclear tracks = N. Original magnification 22,500.

defined lipoprotein membrane. This limiting membrane isolates within the lysosome approximately twelve hydrolytic enzymes. The function of the lysosome has been envisioned as that of an intracellular gastrointestinal tract. The acid hydrolases are utilized to break down materials taken into the lysosome. These breakdown products may then be utilized by the cell as a whole. The lysosome has a second function and acts as a scavenger, engulfing and presumably digesting intracellular debris. This removal of the debris from within the cell matrix contributes significantly to overall intracellular integrity.

The presence of the hydrolytic enzyme, acid phosphatase, in the lysosome

has been utilized to identify these morphologic structures by cytochemical means. Thus acid phosphatase has been used as a biological tracer. Its demonstration, utilizing modifications of the Gomori technique, has been accepted as evidence that the organelle in question fits the current concept of the lysosome (12).

During the past four years at open heart surgery I have taken several square millimeter samples of tissue from the edge of a right atrial incision. This minor procedure adds nothing to the operation. Thirty seconds after removal,



FIG. 4. Ultrastructure autoradiograph enlarged from original magnification 22,500 (Fig. 3) to 112,500. The two comma shaped nuclear tracks (N) are clearly shown in close association with the H disc area (H) of the A band of the sarcomere.

the heart muscle was placed in osmic acid and diced into 1 millimeter cubes. These cubes were fixed in osmic acid (17) and embedded in methacrylate or Epon 812. Sections were cut using glass knives and a Servall Porter-Blum microtome, and viewed in an RCA EMU 3C electron microscope. No additional staining other than that produced by the osmium tetroxide was utilized.

It soon became apparent to me that there were certain dense bodies in the myocardial cells which were very similar, morphologically, to the



Fig. 5. An electron autoradiograph showing nuclear tracks over A band area of myofibrils. One track is in close association with a Z line while two other tracks are in the A band area. The silver crystals here are more filament like in appearances. Z line = Z; secondary A band = A; mitochondria = M; nuclear track = N. Original magnification 11,000.

organelles previously designated in other tissues as lysosomes (pinosome, phagosome, cytosome, dense body) (12). The myocardial lysosomes (Fig. 6) are most commonly round or oval, having an average diameter of approximately 0.5 to 1.5 microns. They are bounded by a unit membrane and contain an overall globular or coarsely granular, moderately dense osmiophilic material surrounding one or more irregular less dense areas. The less dense areas are frequently located near the center of the lysosome. In addition,



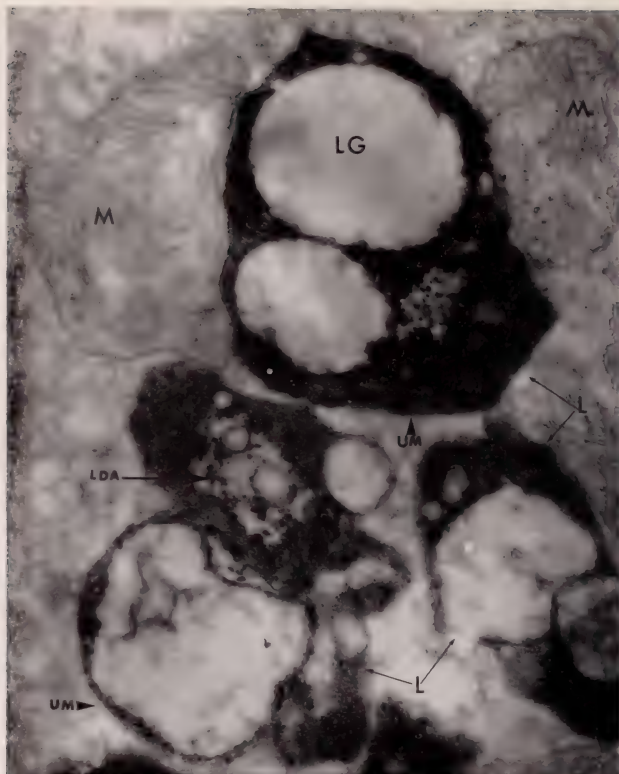


FIG. 6. An electron micrograph of right atrial muscle from a 40 year old white female with mitral stenosis showing characteristic myocardial lysosomes. Lysosomes = L; less dense area = LDA; large globules = LG; mitochondria = M; unit membrane = UM. Stained with VOSO<sub>4</sub>, × 30 minutes. Original magnification 19,700.

there are large globules, faintly osmiophilic which are bounded by a definite membrane. These globules vary in numbers and size within the lysosome and are frequently adjacent to the outer wall of the main body of the lysosome. Although these lysosomes are very commonly located near the nuclear poles of the myocardial cell, they are frequently found with no apparent relationship to the nuclei. They may be found along the sides of nuclei, in distant portions of the sarcoplasm between myofibrils, or disbursed among



the mitochondria (Fig. 7). The appearance of these organelles was so strikingly similar to many of the lysosomes previously described in the literature that I felt that they must be lysosomes. The next step was to see if we could demonstrate the presence of acid phosphatase in the organelles in question.

Using a modified Gomori technique (18, 19) heart muscle was prepared

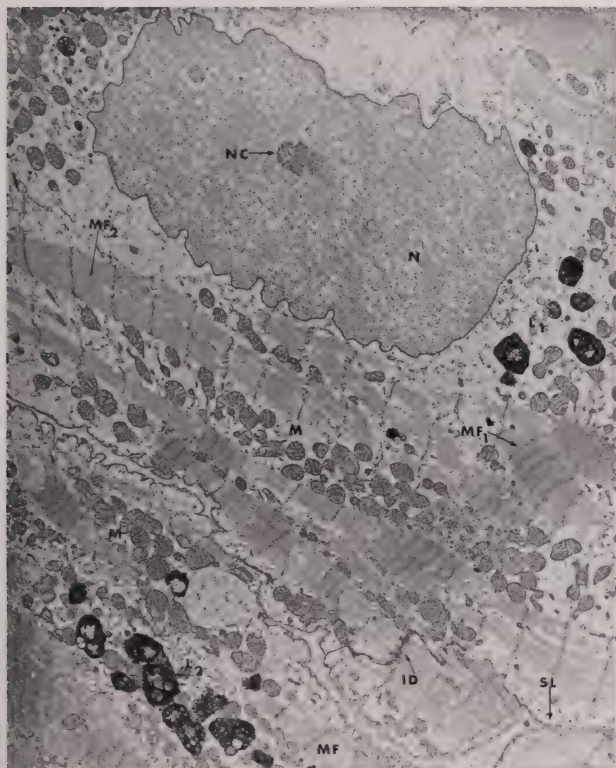


FIG. 7. An electron micrograph of right atrial muscle from a 24 year old white female with mitral stenosis. Lysosomes are shown at a nuclear pole  $L_1$  and also interspersed among mitochondria and myofibrils without relation to a nucleus =  $L_2$ . Lysosomes =  $L_1$ ,  $L_2$ ; nucleus = N; nucleolus = NC; mitochondria = M; myofibril contracted =  $MF_1$ ; myofibril relatively relaxed =  $MF_2$ ; sarcolemma = SL; intercalated disk = ID. Original magnification 11,300.

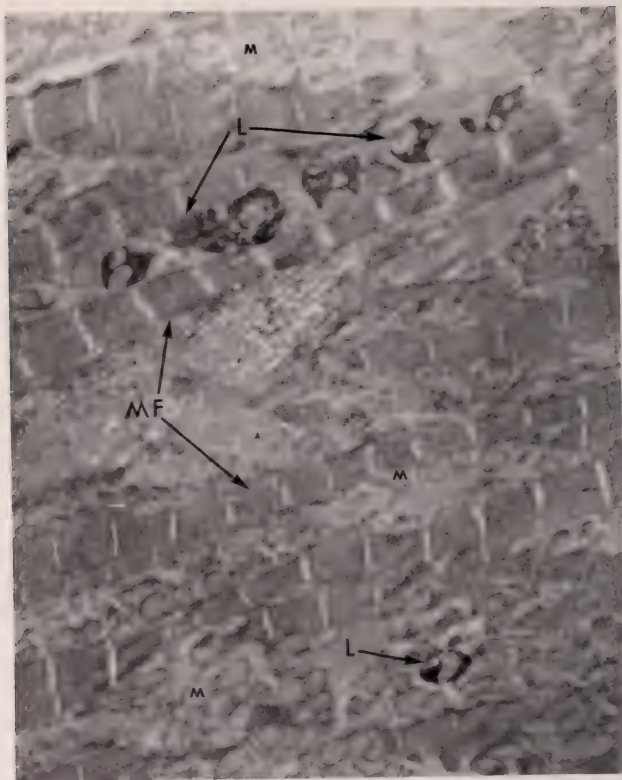


FIG. 8. An electron micrograph of right atrial muscle from a 40 year old white female with mitral stenosis. Tissue fixed in formaldehyde, stained with modified Gomori (18, 19) only. Lysosomes showing precipitated lead = L; myofibrils = MF; mitochondria = M. Original magnification 4,100.

and studied to see if acid phosphatase could be identified by the precipitation of lead in the organelles. The organelles shown in Figures 8 and 9 are similar to the myocardial lysosomes in Figure 6. These organelles (Figs. 8 and 9) do contain precipitated lead. Therefore, presumably, they contain acid phosphatase and fit into the current concept of the lysosome.

While looking at large numbers of heart tissue samples, it soon became apparent that some myocardial cells contained more lysosomes than others.

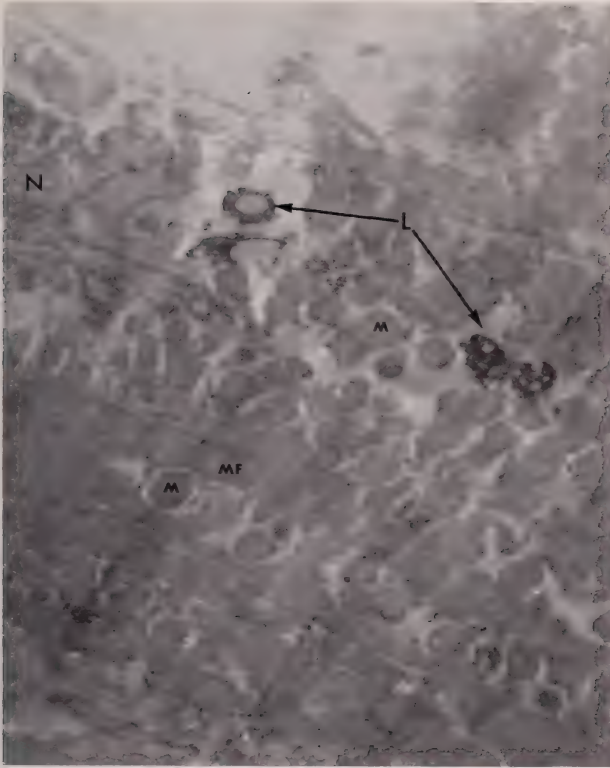


FIG. 9. An electron micrograph of right atrial muscle, 40 year old white female with mitral stenosis. Fixed formaldehyde, Gomori (18, 19) stain only. Lysosomes with precipitated head (L) are shown lying in a characteristic position near a nuclear (N) pole. Mitochondria = M; myofibril = Mf. Original magnification 8,100.

Initially I felt that I could almost identify a particular heart tissue as belonging to a patient with mitral stenosis because of the relatively large number of lysosomes contained in the right atrial section. Also, many times, these lysosomes in patients with mitral stenosis appeared to be what might be called giant lysosomes because of their large size when compared to those found in other heart tissue. Furthermore, while looking at tissue from younger patients with congenital heart disease, it also became apparent that younger

patients generally had fewer myocardial lysosomes than older patients or those with long-standing severe heart disease. As a result lysosome counts were performed in various categories of patients with heart disease to see if there was any correlation between the severity of the heart disease and the number of lysosomes present in the myocardium.

The immediate problem encountered in trying to correlate this material was to depict the degree of the severity of the heart disease as a number easily

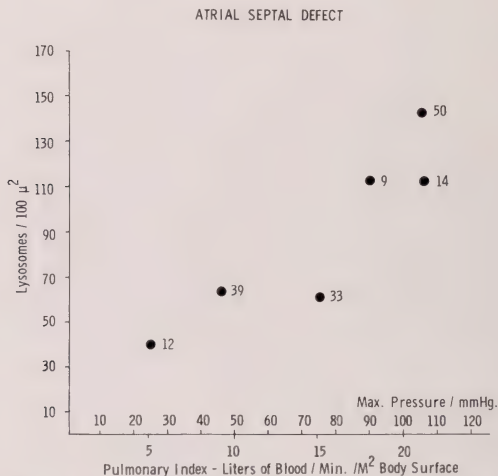


FIG. 10. Graph showing the increase in the number of lysosomes in the right atrial muscle cells relative to the severity of the atrial septal defect as measured by the pulmonary index or degree of right ventricular hypertension. Each dot represents one patient and the number the patient's age. Dots number 9 and 50 represent the two patients with right ventricular hypertension.

used on a graph. This was done in an arbitrary manner. In a group of patients with atrial septal defects of the foramen ovale type the pulmonary index has been used as a measure of the severity of the heart disease. Thus, in four patients with atrial septal defects without pulmonary artery hypertension, the pulmonary index (liters of pulmonary blood flow per minute per square meter of body surface area) has been used as a measure of the severity of the atrial septal defect. There were two additional patients in this series who had an elevated right ventricular pressure or pulmonary artery pressure. In these two patients the highest pulmonary artery or right ventricular pressure (in millimeters of Hg) was utilized as an index to the severity of the heart disease.

If the pulmonary index or the maximum right heart pressure is plotted against the number of myocardial lysosomes per hundred square microns, there is an almost linear increase in the number of lysosomes (Fig. 10). Thus, it appears from this material, that the more severe the atrial septal defect, the more lysosomes present in the individual myocardial cell of the right atrium. Note that this is not just another way of showing that the number of lysosomes increase with age. One of our highest lysosome counts (112 100 square microns) was obtained in a patient only 14 years old. Her pulmonary

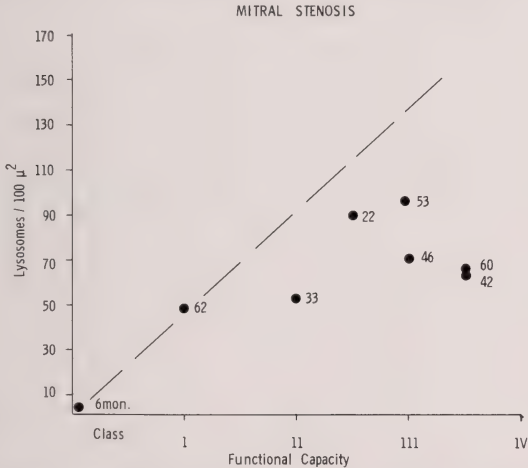


FIG. 11. Graph showing the increase in the number of lysosomes in the right atrial muscle cells of patients with mitral stenosis relative to the severity of the heart disease as measured by the functional capacity. Each dot represents one patient and the number the patient's age. The two dots directly on the line are patients without known heart disease.

index (21 L min  $M^2$ ) is one of the largest left-to-right shunts recorded by our group. This is a much higher lysosome count than was recorded in two older patients (30 and 39 years) who had smaller  $L \rightarrow R$  shunts (Fig. 10).

The next series of patients which we studied were those with mitral stenosis. Here, as a measure of the severity of the heart disease, I have used the functional capacity (Class I-IV) as described by the New York Heart Association. In doing this, one of our cardiologists graded each patient's functional class without knowledge of the lysosome counts. The functional class was then plotted against the number of lysosomes 100 square microns (Fig. 11). The limited data do not allow any positive statement, but again there is the definite suggestion that an increasing severity of heart disease is corre-

lated with an increase of lysosomes in the myocardium. The two points shown directly on the line are two cases (6 months and 62 years) without heart disease in whom right atrial sections and lysosome counts were done. There were essentially no lysosomes found in the patient 6 months of age, whereas the patient aged 62 years with no heart disease showed a definite increase in the number of lysosomes. Still, the number of lysosomes was not as great as found in patients that age or younger with heart disease.

As to what this means, I assure you, I do not know at the present time. Several interesting speculations can be made. First of all, we have shown that lysosomes are present in significant numbers in human heart muscle, just as they are present in most of the other animal cells that have been studied. These organelles are lysosomes according to the present day concept because they are morphologically similar to those described in other tissues and because they contain acid phosphatase. Finally, although the significance is certainly not known, it appears that lysosomes increase in number in the right atrial tissue of patients with heart disease and that this increase in number correlates directly with the severity of the disease. If these organelles are truly the scavengers of the cell, maintaining the integrity of the cell by removing debris, then the increased number of lysosomes present in individuals with heart disease may mean that more work has to be done in the muscle cell undergoing stress as a result of congenital and acquired heart disease.

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# Considerations in the Surgical Treatment of Transposition of the Great Vessels

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Transposition of the great vessels must be defined before it is discussed in detail because of the variety of anomalies which may be associated with it. Some authors include them, and the resulting combinations give many variants in the transposition complex. Lev has given a useful list which simplifies this matter and should be referred to in questions of associated anomalies which may be encountered with transposition (1). We limit the term "transposition" to hearts that have two ventricles with aorta and coronary artery branches originating from the right ventricle and pulmonary artery from the left. The relations of vena cava and pulmonary veins to the heart are normal. Also, the tricuspid and mitral valves open normally and are found in the right and left ventricles, respectively.

The relative importance of transposition of the great vessels within the group of congenital cardiovascular malformations has been stressed in several reports. The clinical course and usual dismal prognosis have been clearly outlined also. We will not describe them, but rather will review anatomic and physiologic features of importance in selection of transposition patients for operation. Emphasis will also be given to points which influence choice of operation for individual cases.

The principal anatomical features of interest in consideration of operations for palliation or cure of the anomaly are, first of all, some extracardiac details. The aorta lies in front of and, in the majority of cases, slightly to the right of the pulmonary artery; the valves at the origins of these two vessels are on the same horizontal plane (2). It is convenient, viewing the base of the heart from the front with it in situ, to name the aortic sinuses, right, left, and posterior. The right coronary artery originates in the posterior sinus, and the left artery in the left sinus. The ductus arteriosus is patent in at least half the cases, and an aortic septal defect is another possibility but it is a very rare occurrence. Important features of intracardiac anatomy are summarized as follows. Communication of atria through their septum is present in virtually all cases. In only about one-half of them, however, is there a true defect in the fossa ovalis. Formation of the ventricular septum has been studied in considerable detail, and defects in it have been found in at least 30 per cent of specimens or more, depending upon the criteria for inclusion of cases in the transposition category (1, 2). The defects are located at the base in most instances and only rarely are found in the septum closer to the apex. Elliott's report also noted the occurrence of a

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small number of left ventricular-right atrial communications in their series. Clefts in the septal leaflet of the tricuspid valve have been observed as well. The last comment on intracardiac anatomy concerns the ventricular outlets. There is, in a small proportion of the cases, obstruction to flow into the aorta due to hypertrophy of the crista supraventricularis. Aortic valvular stenosis, however, has not been encountered, though a bicuspid valve has been seen in a small number of instances. Subvalvular stenosis is also encountered in the left ventricle and obstruction at the pulmonary valve is an associated malformation which is not rare.

The course of the circulation varies considerably in patients with transposition depending primarily upon associated cardiovascular malformations and the development of arteriolar narrowing in pulmonary vascular bed. Patients with small communications at the atrial level and with intact ventricular septum have virtual separation of the systemic from the pulmonary circulation. Their intra-uterine life is affected very little, however, because of the resistance to flow through the unexpanded lungs and patency of the ductus. Expansion of the lungs after birth and reduction of resistance in the pulmonary bed results in flow from aorta through ductus into the pulmonary artery. The course thereafter is an isolated circuit through the capillaries and pulmonary veins, into the chambers of the left heart, and out the pulmonary artery. Flow from the aorta through ductus into the pulmonary artery leads to elevation of pressure in it and the left ventricle and, eventually, pulmonary hypertension with absence of gradient between aorta and pulmonary artery. Marked elevation of pulmonary artery pressure may even reverse the shunt through the ductus. Flow away from the pulmonary circuit through the ductus leads to increased venous return to the right atrium. This, in turn, through elevation of right atrial pressure may lead to shunting into the left atrium through the foramen ovale. The effect of this is to increase the flow through the left heart and pulmonary artery, and so perpetuate the shunt through ductus into aorta. There is admixture of blood from the systemic and pulmonary circuits by this mechanism while ductus and foramen ovale remain open. Their closure results in death of the infant within a period of weeks. Conversely, a gross defect in the atrial septum permits free admixture of blood from the two circuits; this theoretical advantage to patients has been borne out by clinical observation (3). As suggested in the discussion so far, pathways which allow admixture of blood between the two circuits are of crucial importance to the life of the patient with transposition. Ventricular septal defects permit the streams of the two circuits to cross to some extent. Yet it is impossible to predict accurately the effect upon hemodynamics in every case. There is ample confirmation that a left-right shunt at ventricular level and pulmonary stenosis is a combination with relatively good life expectancy. Defects which permit shunting at the ventricular level may result, as Noonan has shown, in excessive pulmonary blood flow (4). Congestive heart failure with early death is the result of this hemodynamic pattern. Development of pulmonary vascular obstruction is seen in

a considerable proportion of patients with ventricular septal defects. The shunt in these cases may be in both directions at the ventricular level. However, it is right-left at the atrial level as pulmonary blood flow decreases due to progressive changes in the vessels of the lung.

Detailed discussion of the clinical picture presented by patients with transposition is outside the scope of this report. The important points, however, should be reviewed briefly. Cyanosis is striking, as a rule, but varies indirectly with degree of interchange of blood between systemic and pulmonary circuits. Therefore, it is possible during the neonatal period, at least, for some patients to have relatively good color. Development of polycythemia and clubbing are associated with this sign. Difficulty with feeding is outstanding in the majority of cases; weight gain and bodily development are subnormal. Respirations are abnormally fast, as a rule, and after a few months of life, increase in antero-posterior diameter of the chest is apparent. There are no diagnostic signs from the cardiovascular system. Thrills over the precordium and murmurs have limited value. The second sound at the base at the left of the sternum is abnormally loud because of proximity of the transposed aorta. Congestive failure is common, and with this develop the signs of pulmonary congestion (unless there is associated pulmonic stenosis), engorgement of the liver, and peripheral edema.

Further investigation with electrocardiography, routine x-rays, cardiac catheterization, and angiocardiology is required to establish the diagnosis. Right axis deviation and right ventricular hypertrophy shown by the precordial leads are the pattern often described as characteristic of transposition. Recently, however, Elliott's analysis of a group of patients showed that electrocardiographic evidence of biventricular hypertrophy was found in patients who had either a large patent ductus or a large ventricular septal defect in association with transposition (5). Chest x-rays have considerable diagnostic value because of demonstration of the following points. The size of the heart is normal at birth, but the majority of cases show striking enlargement within a few months. There is usually a concavity in the upper border of the silhouette left of the sternum. The shadow of the great vessels is narrow when viewed from the front and relatively wide when viewed from the left anterior oblique. Finally, and of considerable importance, are the vascular markings in the lung fields. They are normal or slightly increased during infancy and become progressively more prominent in childhood. Cardiac catheterization demonstrates systemic pressure in the right ventricle and absence of systolic gradient between the chamber and the aorta. The pulmonary artery is entered in a high proportion of cases with ventricular septal defect, and blood samples taken there have a higher oxygen content than those taken from the aorta. In addition, unless there is pulmonic stenosis, there is some degree of pulmonary hypertension in every case. Angiocardiograms made in lateral view will establish the diagnosis by opacification of the right ventricle and its outflow into the aorta which occupies a grossly abnormal anterior position.

There are at least three other conditions to be eliminated when considering the diagnosis of transposition. The first is tetralogy of Fallot. Chest x-rays show increased pulmonary vascularity with transposition in contrast with decreased vascularity with tetralogy. Further, the heart enlarges progressively with transposition, but remains relatively small in cases of tetralogy. It must be added, however, that differentiation of transposition with pulmonic stenosis from tetralogy may be impossible without satisfactory angiocardigrams. The second condition of importance in the differential diagnosis is truncus arteriosus. Enlargement of the heart is characteristic of this condition as well as transposition. Prominence of the aortic knob distinguishes truncus, however. Patients who survive to childhood have a continuous murmur if truncus is present, but this sign does not develop if the malformation is transposition. Pulmonary stenosis is the third condition to be distinguished from transposition. Infants with this deformity may have congestive failure in the neonatal period and cyanosis may be present due to right-left shunting through the foramen ovale. The picture of a baby with pulmonic stenosis is different, however, because the second sound at the base, left of the sternum, is weak or absent. His course under treatment with digitalis is better, cyanosis diminishes, and chest x-rays eventually show fullness of the pulmonary conus due to post-stenotic dilatation. These changes do not take place, or are much less striking with transposition.

Results of surgical treatment of transposition of the great vessels are acceptable if viewed with proper reference to the complexity of the malformation and the natural course of untreated patients. Our purpose in this report is to describe long-term effects of one type of palliative operation on two patients, and to summarize results of a second type of palliative procedure in four patients with a two-year follow-up. We are unable to draw conclusions about the relative merits of all palliative operations under trial at present; the Baffes (6) procedure, for example, has given excellent results but we have had no experience with it. Valuable comparisons of this type must be made in the future from a large number of cases in which there is accurate classification according to anatomical variants and their effect upon hemodynamics. It is our purpose in this report also to describe the case of a 10 week old baby who was cured with a Senning operation.

#### EXCISION OF THE ATRIAL SEPTUM AND CONSTRICTION OF THE PULMONARY ARTERY

There have been numerous suggestions in surgical literature, since original publication of Blalock and Hanlon (7), that creation of an atrial septal defect would give palliation for transposition patients. We believed that those in the transposition group who had ventricular septal defect without other associated deformity might benefit more if, in addition to creation of an atrial defect, there was produced obstruction to the outflow of the left ventricle. Our hypothesis was that as resistance was increased to flow from the left ventricle into the pulmonary artery, a larger volume of blood would shunt through the ventricular septal defect into the aorta. Muller and Dam-

man (8), in 1952, had reported the value of surgical pulmonary stenosis in reduction of pulmonary hypertension. We applied their idea failing to appreciate its value for prevention of the effects of pulmonary engorgement and believing its main influence would be on the shunt at the ventricular level as mentioned.

A synopsis is given from the records of two patients on whom we have the longest postoperative observation.

E.J.McE., Children's Hospital No. 90858, was very cyanotic in his first two years and growth was retarded. Electrocardiogram showed right ventricular hypertrophy. X-rays demonstrated cardiac enlargement and increased pulmonary vascularity. An angiocardigram proved transposition and ventricular septal defect. In July 1954, a large part of the atrial septum was excised under direct vision during hypothermia and inflow occlusion, and the greatly dilated pulmonary artery was constricted to a diameter of 8 mm with a silk ligature. Improvement was apparent within a few months. Evaluation nine years after operation, at this writing, showed that he was mildly cyanotic and had fair exercise capacity. He was at the 75th percentile for height and 25th for weight. Hemoglobin was 18.7 Gm% and hematocrit was 55%. Moderate cardiac enlargement persisted, as well as prominence of the vascular markings in the lungs. The electrocardiogram showed P mitrale, first degree heart block, and right ventricular hypertrophy. A shunt through the atrial defect was proved by catheterization and there was marked pulmonic stenosis.

R. C. S., Children's Hospital No. 94043, had cyanosis and rapid respirations shortly after birth. Electrocardiogram showed right ventricular hypertrophy. Heart size was normal, but pulmonary vascularity was increased. An angiocardigram established the diagnosis of transposition. Congestive failure developed, and at nine months he was cachectic and barely able to move. In February 1955, part of the atrial septum was excised under direct vision with hypothermia and inflow occlusion and the tense pulmonary artery was constricted to a diameter of seven mm with braided silk ligatures. Right hemiparesis developed on the ninth postoperative day. Evaluation eight and one-half years after operation showed considerable improvement, but he was still moderately cyanotic. Mental retardation and residual effects of hemiparesis restricted him considerably. He was below the third percentile in both height and weight. The hemoglobin was 20.5 Gm% and hematocrit 74%. There was moderate cardiac enlargement and some prominence of pulmonary vascular markings. The electrocardiogram showed marked right ventricular hypertrophy. Angiocardiography demonstrated a large atrial septal defect, a ventricular septal defect, transposition and pulmonic stenosis.

The results of cardiac catheterization are given in Table I.

There are several points to be considered in evaluation of this form of operative treatment. First, our total experience is comprised of ten cases with seven deaths in the group. We believe improved selection of patients and better control of technical factors in the operation will reduce this high mortality rate to an acceptable level. Second, the clinical improve-



ment obtained has been good enough to warrant further trial of the operation. In this connection, reference should be made to Toole's (9) experience with the simpler operation of creation of atrial septal defect. We believe that although the general result has been good in only one (E. McE.) of our two, prevention of pulmonary hypertension was accomplished in both. (We have not reported our third survivor because of an inadequate follow-up period. Twelve months, at least, must elapse from the date of operation before its effects can be assessed.) Finally, alterations in the atrial septum and main pulmonary artery by this method are not so complicated that a curative operation would be impossible later. Advances in cardiac surgery

TABLE I

*Results of Catheterization of Two Patients 9 and 8½ Years After Creation of ASD and PA Constriction*

	Pressure mm Hg		Per cent O <sub>2</sub> Saturation	
	E. McE.	R. S.	E. McE.	R. S.
Superior vena cava				
Low.....			66.1	46.7
High.....			66.1	51.1
Right atrium				
Low.....			75.2	52.0
Mid.....	8/2	2/0	89.3	55.4
Right ventricle.....	110/-15 to +8	139/-20 to +10	92.0	85.1
Aorta.....		102/65	85.1	62.2
Left pulmonary vein.....		2 (wedge)		98.2
Left atrium.....		2/-1		97.9
Left ventricle.....		102/-10 to 0		91.4
Pulmonary artery.....	9/3		97.1	85.7
Cardiac index.....	3.4	4.1		
Left-to-right shunt.....	15.1 L/min	2.9 L/min		
Right-to-left shunt.....	1.5 L/min	2.4 L/min		

may, within a few years, cure a certain proportion of transposition patients if the pulmonary vascular bed is free of arteriolar disease.

#### ANASTOMOSIS OF THE SUPERIOR VENA CAVA TO THE RIGHT PULMONARY ARTERY

Many patients with transposition who fall in the subgroup with pulmonic stenosis have been treated with either a Blalock or Potts anastomosis. In our experience, however, the benefit immediately following operation was not predictable. The long-term effect was not known either, but some skepticism developed as we observed patients who had had these operations for other cardiac malformations. We decided, therefore, to use the anastomosis of superior vena cava to right pulmonary artery, described by Glenn (10), on patients in this special category (i.e., transposition with ventricular septal defect and pulmonic stenosis).

Criteria for evaluation of the effect of operation after one or two years are given in Table II. The first patient, P.D., had a functioning Blalock anastomosis which was made at the age of two. An unusual feature in her case also was unexplained systemic hypertension which has not recurred since the Glenn anastomosis. The last patient, C.D., developed the postpericardiotomy syndrome (11) in the third postoperative month. Review of electrocardiograms and chest x-rays shows no significant change in this group. It is apparent that results to date are very good and with a very low operative risk they are far better than obtained by any other palliative procedure for this condition. Their excellence, in fact, suggests another plan of surgical treatment. It might be more beneficial to band the pulmonary artery in those

TABLE II  
*Glenn Anastomosis in Patients with Transposition, VSD, and PS*

	P. D.		E. K.		W. C.		C. C.	
	Before	After	Before	After	Before	After	Before	After
Cyanosis	++++	—	+++	+	+++	0	++++	—
Exercise capacity	very limited	near normal	very limited	near normal	very limited	normal	very limited	normal
Hemoglobin, Gm%	20.5	18	19	14	19	14	22	17
Hematocrit, vol%	68	57	60	43	60	40	73	54
Follow-up period, months	24		18		18		12	
Age at operation, years	6 $\frac{1}{2}$		7 $\frac{1}{2}$		2 $\frac{1}{2}$		10 $\frac{1}{2}$	

with transposition and a simple ventricular septal defect and carry out cavo-to-pulmonary artery anastomosis rather than excision of atrial septum.

#### INTRA-ATRIAL CORRECTION

The principle given by Albert (12), in 1954, for correction of transposition by redirection of caval and pulmonary venous flow at the atrial level has been the basis for several new operations. It is beyond the scope of this report to review them, but credit must be given to Senning (13), Barnard (14), Shumacker (15), and Kirklin (16) for its application and the first curative operations for transposition. Senning's and Barnard's patients were nine and sixteen years of age, respectively. Shumacker's patient was one year of age, and Kirklin was successful with three infants. The record of our only patient to date is summarized as follows.

J.E.S., Children's Hospital No. 150721, was cyanotic at birth. There were frequent episodes of paroxysmal dyspnea and cyanosis relieved by morphine

and oxygen. Cardiac failure was partially controlled by digitalis and diuretics. Electrocardiogram showed P pulmonale and marked right ventricular hypertrophy. The heart was moderately enlarged by x-ray and pulmonary vascular markings were increased. Cardiac catheterization was not complete, but pressure in the left ventricle, entered via atrial septum and mitral valve, was only one-half systemic pressure. An angiocardigram confirmed the diagnosis and gave additional evidence against a ventricular septal defect. She was operated upon in March 1963, in her tenth week of life. She weighed seven pounds and three ounces. Deep hypothermia was induced during perfusion to permit total interruption of circulation for 35 minutes. This was the time required for closure of the patent ductus and completion of the intraatrial portion of the operation (Figs. 1-3). Perfusion was resumed, and after rewarming she did not require further support. Arterial oxygen saturation, postoperatively, varied between 91 and 95 per cent. An angiocardigram on the thirteenth postoperative day confirmed the normal course of her circulation, and she has progressed as a normal baby to date, nine months after operation (December 1963).

We believe intra-atrial correction should be performed if the child's inter-ventricular septum is intact and a serious degree of pulmonary hypertension has not developed. The dilemma at present is formed by inherent hazards of open-heart operations on children under one year, on the one hand; and on the other, development of pulmonary hypertension during delay for the child to grow.

#### CONCLUSIONS

The cardiac malformations associated with transposition of the great vessels form the basis for division of patients into subgroups. Choice of operation should depend upon the hemodynamics of the subgroup and the operative procedure selected which will improve it most. Clearly, there is not enough experience to form a plan which will serve as a guide for every case. We believe, however, that it is useful, even at the present stage, to follow this set of rules.

1. The child with transposition, an intact ventricular septum, and left ventricular or pulmonary artery pressures significantly lower than systemic pressures should have closure of the ductus arteriosus and intra-atrial correction as described by Senning.
2. The child with transposition, ventricular septal defect, and normal left ventricular outflow should have closure of the ductus arteriosus, excision of the atrial septum, and banding of the pulmonary artery.
3. The symptomatic older child with transposition, ventricular septal defect, and pulmonic stenosis with or without a previous Blalock anastomosis should have anastomosis of the superior vena cava to the right pulmonary artery (Glenn). However, the Blalock or Potts procedure is preferred in infancy.
4. Pulmonary arteriolar disease with marked elevation of vascular resistance is a contraindication to any of these surgical procedures.



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## Carbon Dioxide, Waste Product or Elixir?

FRANK GOLLAN, M.D.\*

As we set out to sail uncharted seas in medicine it is well to reaffirm our faith and hope to relate our biological destiny as well as our work to nature around us. The guiding star in this uncertain voyage is the knowledge of naturalists who have lived, labored and died before us. In the field of extracorporeal circulation in particular, we should never forget that the principles of circulating, oxygenating and dialyzing of blood outside the body have been presented to us at the turn of the last century and there is no reason to be stupefied by the current plethora of bigger and better machines. We should use these ingenious devices first of all to relieve human suffering and then to explore the physiological conditions which will finally make their use unnecessary by inducing in man this miraculous creation of nature, hibernation.

At a meeting of the Royal Society of Philosophy in London Dr. Marshall Hall said on March 1, 1832: "In the hibernating animal the respiration is nearly suspended. Had not the irritability of the heart become proportionately augmented, the actions of life must have ceased. There is no stagnation of blood at the heart, consequently no uneasiness and the animal becomes more and more lethargic as the circulation of venous blood is more complete. This lethargy is eventually interrupted by circumstances which break ordinary sleep as external stimuli and the cause of appetite" (1). This first description of hibernation is a clairvoyant outline of our final aim to produce in man a condition not requiring anesthesia, artificial respiration, ice immersion, and "heart-lung" machines. In hibernating animals the heart rate begins to drop before the body temperature shows any change, but then the heart speeds up again and by shivering the animal regains its temperature. After a few "test drops" of progressively lower temperature, the nervous system seems to be conditioned for the final plunge to the lowest temperature which is always slightly higher than the environment. The animals are able to reset their thermostat to a varying gradient of the outside temperature without awakening, but wake up temporarily to have a snack. During arousal in spring the heart rate may accelerate to a rate a hundred times faster than during hibernation and after about three hours of violent shivering the animal is evenly rewarmed and fully awake. This feat of harmonious autonomic adjustment cannot be accomplished by any nonhibernating mammal, including man, because as the heart rate, the cardiac output and the circulation time slow down, the cold blood becomes too viscous and capillary flow ceases; the heart shows an "uneasiness," as Dr. Hall would call it, by becoming irregular and death ensues by ventricular fibrillation.

By far the largest part of the vascular bed is part of the venous tree, par-

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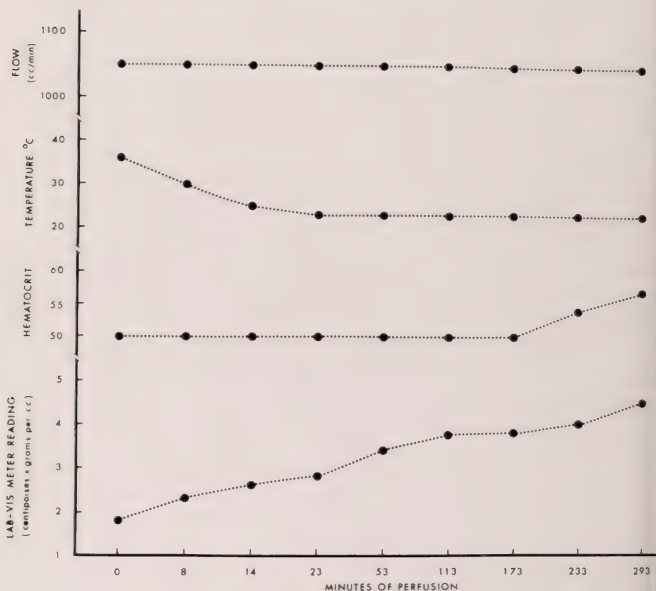


ticularly the huge reservoir of venules where the viscous blood finds its resting place. It would require a much more forceful and faster circulation of much less viscous blood to provide the tissues with oxygen. Since the animal lacks a rapid and forceful heart, as well as enough thin blood, it is quite obvious that the temporary loan of a spare pump and some watered-down, oxygenated blood would be a great help in this time of need. In fact, man's best friend is so grateful for this mediocre gift and the professional courtesy extended to him, that he permits us to get all his lazy and thick blood back into the circulation again and he rewards us by tolerating a much lower temperature and longer period of circulatory arrest. The heart rate and temperature curve of dogs cooled by blood perfusion is similar to the one we see in the hibernating animal.

Is it really as simple as that? Do the mechanical devices re-establish adequate capillary flow at almost freezing temperatures? There is no doubt that they have improved the circulation of the internal organs supplied by large arteries, as evidenced by the pronounced temperature gradient and the seemingly unimpaired function of kidneys, liver and heart. The heart is mentioned last because it is farthest from the femoral artery cannula returning oxygenated cold or warm blood to the body. In contradistinction to the normal condition where the heart and the brain are the first to receive blood from the large outflow tract of the left ventricle and the ascending aorta, now they are last in line and are expected to be satisfied with the dissipated leftovers of the thin jet stream hitting the pool of inert, viscous blood in a broadening aorta. By measuring the available myocardial oxygen it can be shown that the heart receives more coronary flow if the same volume of blood is returned into the left subclavian, than into a femoral artery. The heart can tolerate a lowered coronary flow under such conditions, but the integrity of the brain may be impaired. It is particularly distressing to read reports where "informed guesses" from oxygen consumption curves derived from low-flow and short-term experiments have led to long operations with cessation of the circulation, resulting in severe brain damage in children. A review of the literature of hypothermic perfusion indicates that there are three physiological factors which guarantee success of this procedure. They are: large blood flow; blood dilution; and increased carbon dioxide tension.

High blood flow does not only depend on the continuous drainage of venous blood through large bore catheters, but also on the viscosity of blood. The viscosity of the blood goes up about 5 per cent per degree centigrade of lowered temperature, and finally it becomes so thick that it cannot be aspirated into a syringe. It would be desirable to measure viscosity while blood is flowing and not in a test tube, because blood is a so-called non-Newtonian fluid. This term simply means that if blood is stagnating, the particles will settle and viscosity will go up, whereas if you keep it moving the viscosity will be lower (2). Therefore, the viscosity of blood should be measured while blood is flowing. Unfortunately this cannot yet be done intravascularly and therefore we have to be satisfied by inserting an ultrasonic probe into the extracorporeal circuit. By continuous monitoring of blood viscosity we can study the effects of hemato-

erit, temperature and velocity. The initial changes of hematocrit by blood dilution and temperature will result in the final measured viscosity (Fig. 1). But, if we stop the flow in the extracorporeal circuit we also can measure the effect of velocity. This effect is very small and does not lower viscosity to the extent hemodilution does (Fig. 2).



#### CONTINUOUS VISCOSITY MEASUREMENTS OF FLOWING BLOOD

Fig. 1 Continuous monitoring of extracorporeal blood viscosity by an ultrasonic probe.

During a perfusion we still would like to know whether the brain, the most exacting customer of oxygen, is always satisfied. Up to now we did not have any means of instantaneous and continuous measurement of blood flow to the brain during a perfusion. The nitrous oxide extraction and radioisotope dilution methods do not lend themselves for such a procedure because they are much too cumbersome and time consuming. We have found that electric impedance plethysmography does fill that need. This forbidding name hides a simple principle. With the ebb and flow of blood to and from the brain, electrical current flow between two electrodes is affected. Just like in the hemo

dynamic system, resistance equals pressure over flow, or we can state in electrical terms that impedance equals voltage over current. Electrical impedance accompanying changes of pulse volume have been explored since the beginning of the century; however, cranial plethysmography or rheoencephalography (3) has suffered from an abundance of theoretical considerations and premature

### THE EFFECT OF VELOCITY ON BLOOD VISCOSITY

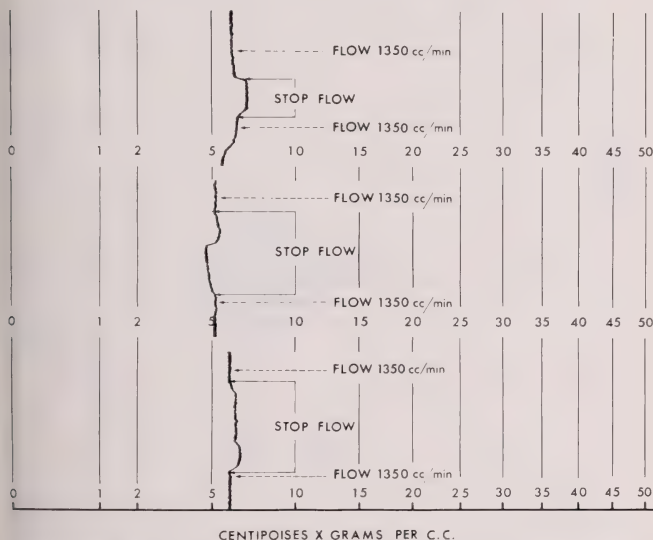


FIG. 2. Effect of velocity on viscosity of extracorporeal blood during hypothermic perfusion.

clinical applications on the one hand, and a lack of controlled physiological experimentation on the other.

A so-called heart-lung machine is ideally suited to bypass the anatomical labyrinth of the cerebral circulation in the dog and to perfuse the isolated head and brain in order to analyze and even to calibrate the rheoencephalogram. In such an experiment venous blood is drained from the right atrium of a closed-chest dog into a pump-oxygenator, and the blood is returned to both common carotid arteries (Fig. 3). When partial cardiopulmonary bypass is established the heart is made to fibrillate by transthoracic shock, and an isolated head perfusion through the carotid arteries results, in which flow, pulse pressure and rate, gas content and temperature can be controlled. Lead foil

electrodes in the shape of contact lenses are put on the corneae to measure the electrical impedance or the pulse volume changes. The eyeballs are the only soft parts protruding from the solid skull, and with monopolar electrodes one can measure carotid blood flow changes to both eyes independently. Large lead foil electrodes attached to the skull measure almost entirely blood flow changes in the external carotid arteries of the dog. The simultaneous tracings of the rheoencephalograph, of an electromagnetic flow meter and a pressure transducer can now be analyzed (Fig. 4). In experiments in which either the flow or the pulse rate were kept constant, we were able to correlate directly the height of the amplitudes of the electrical resistance changes with changes

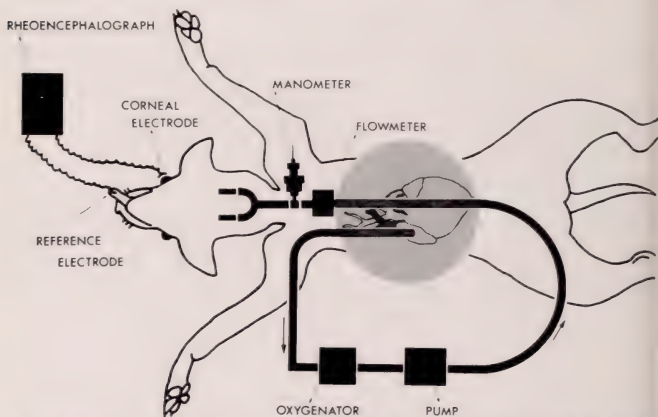


FIG. 3. Controlled perfusion of isolated brain for the experimental analysis of rheoencephalography.

in stroke volume of the pump (Figs. 5, 6). Thus, instantaneous and continuous monitoring of cerebral blood flow may prove to be a most important criterion of adequacy of perfusion.

Gas exchange, the third point mentioned, becomes a highly complicated problem the moment the temperature is changed. There are two distinct and separate factors operative at a lower body temperature: the freely dissolved oxygen in the water of the blood and tissues, and the captive oxygen bound to the pigment of circulating red cells and stationary muscle cells. Let us first consider the dissolved oxygen. As the temperature is lowered, water will contain more dissolved oxygen because the movements of the atoms slow down and the rush to return home to the atmosphere changes into a leisurely pace. The amount of dissolved oxygen of .3 volume per cent at normal body temperature and breathing air rises to as much as 3 to 4 volume per cent if we cool

and oxygenate blood with 100 per cent oxygen (Fig. 7). This is as much as the normal arteriovenous difference in oxygen content and thus at a high flow rate this is actually enough to cover the oxygen consumption of a hypothermic animal. To prove this point we can wash out the red cells and perfuse the animal with plasma and Ringer's solution (Fig. 8). During this period the dog

### RHEOENCEPHALOGRAPH, FLOW AND PRESSURE IN PERFUSED DOG BRAIN

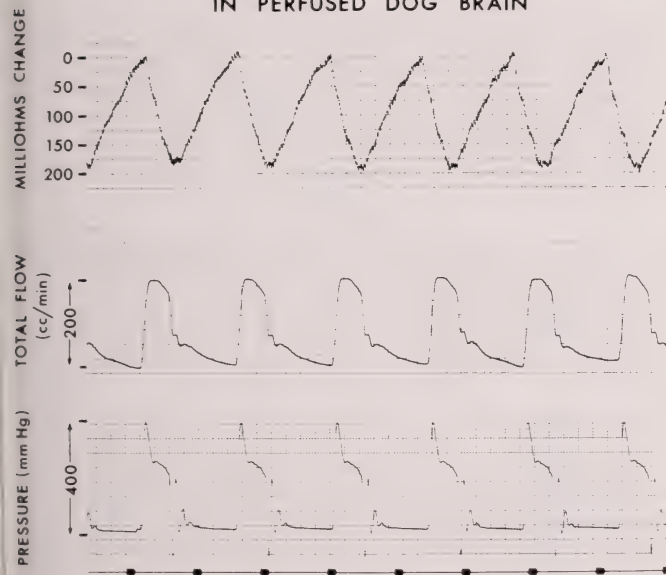


FIG. 4. Simultaneous recording of changes of electrical impedance, flow and pressure in perfused isolated brain of dog. This record of very high amplitudes of all parameters was chosen for better visualization.

resembles the only other creature known to live without any blood pigment—namely, the completely transparent “ice fish” which lives only from the high amount of dissolved oxygen in the cold Arctic waters. Before rewarming the dogs one has to give them the benefit of red cells, and these temporarily bloodless animals have survived without clinical evidence of damage (4). Their oxygen enriched body water kept them alive just like Haldane’s mice under hyperbaric oxygenation whose hemoglobin was put out of circulation

by carbon monoxide (5). It is also possible to transform mice into fish by submerging them in a chamber filled with buffered Ringer's solution under a pressure of 7 or 8 atmospheres of oxygen. It is an amazing sight to see mice swim in an aquarium for a day or so, but one really should not be that astonished because these mice obey Henry's law which states that the weight of a gas absorbed by a given liquid is directly proportional to the pressure of the gas. Thus, during hypothermic perfusion at one atmosphere of oxygen tension and

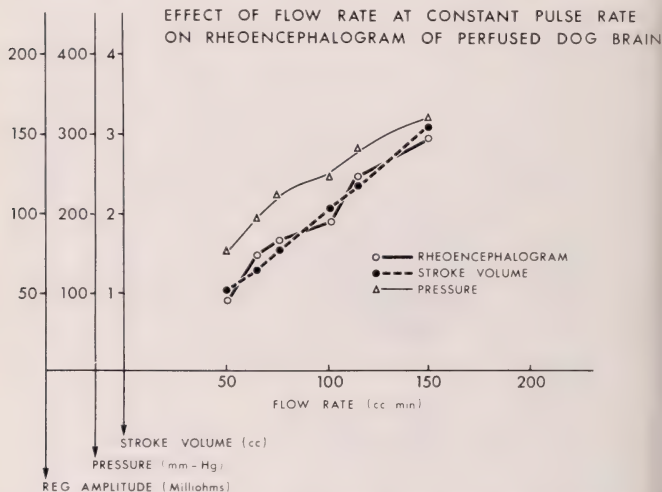


FIG. 5. Effect of changing flow rate at constant pulse rate on rheoencephalogram of perfused dog brain.

during hyperbaric oxygenation the dissolved oxygen in body water can assume biological importance.

The mechanism of oxygen transfer from red cells is of a different nature. Barcroft and King described the effect of temperature on the dissociation curve of hemoglobin, and they found what has been called the "shift to the left" (7). What does this "shift to the left" mean? The bright red arterial blood of the hypothermic animal evokes a sense of false security because the oxygen is glued to hemoglobin but cannot come off. Therefore, we would like to shift or bend these curves back to the right to induce the greedy hemoglobin to release more oxygen to the needy tissues. A famous trio of physiologists, Bohr, Hasselbach and Krogh, have shown that this can be done by increasing the carbon dioxide tension, and by honoring only the first violinist we speak of the "Bohr effect" (8).



We have tried to repeat the work of others who acidified blood by hydrochloric acid. The disadvantage of this procedure lies in the uncertainty of actual penetration of this mineral acid into red cells at a lower temperature and in the necessity of having to neutralize with a base afterwards. But, worse than that, if one tries to acidify highly oxygenated blood coming from an oxygenator the chances of dissociating more oxygen are very poor because the pH effect is minimal at the upper and shallow end of the curve. One has to

### EFFECT OF PULSE RATE AT CONSTANT FLOW RATE ON RHOENCEPHALOGRAM OF PERFUSED DOG BRAIN

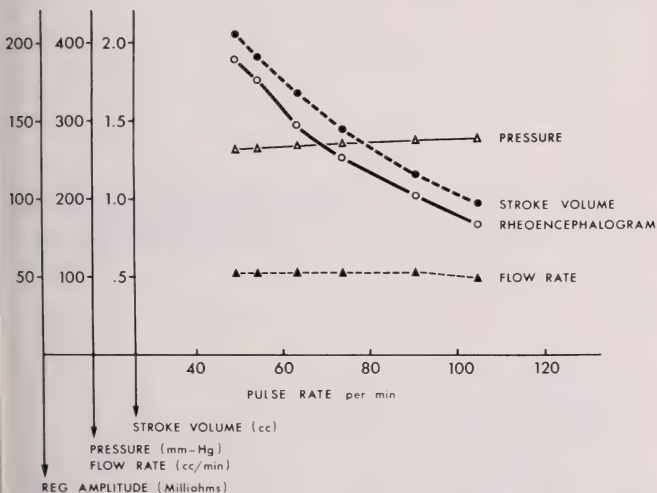


FIG. 6. Effect of changing pulse rate at constant flow rate on rheoencephalogram of perfused dog brain.

test this concept in the venous range of oxygen tension of 20 to 40 mm Hg, where the dissociation does take place in the body. If more oxygen should be made available, it may manifest itself as an increased consumption.

Since it is difficult to produce a steady state by perfusing an animal with venous blood we chose first the simpler isolated heart preparation (9). Dog hearts were perfused with venous blood of an oxygen tension of 20 mm Hg, and diluted hydrochloric acid was added to lower the pH, and THAM buffer was used to raise the pH (Fig. 9). No change in oxygen consumption took place. However, there was a flaw in this experiment because the pH affected the heart rate as well as the myocardial contractile power. In 1913 G. R. Mines

observed that the myocardial contractility of a turtle heart decreased when the pH of the perfusate was lowered and could be restored by raising the pH to its previous level (10). It is discouraging that after fifty years this impor-

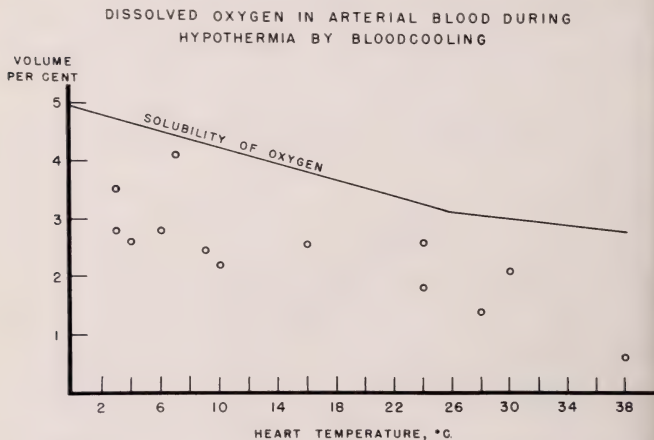


Fig. 7. Effect of temperature on solubility of oxygen in water (solid line) and on oxygen content of arterial blood during hypothermic perfusion with a pump-oxygenator (open circles).

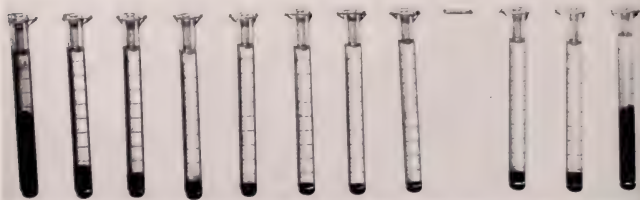


Fig. 8. Hematocrit determinations during hypothermic perfusion without blood.

tant fact still has to be rediscovered by investigators in the laboratory and that it still escapes attention at the bedside. If one suspects a metabolic acidosis at the end of a prolonged perfusion, one can almost titrate the blood pH back to normal by adding THAM buffer and substitute the return of strong contractions for the needle of a pH meter. This dramatic effect is due to the fact that the catecholamines, like noradrenalin, have an optimal pH of about

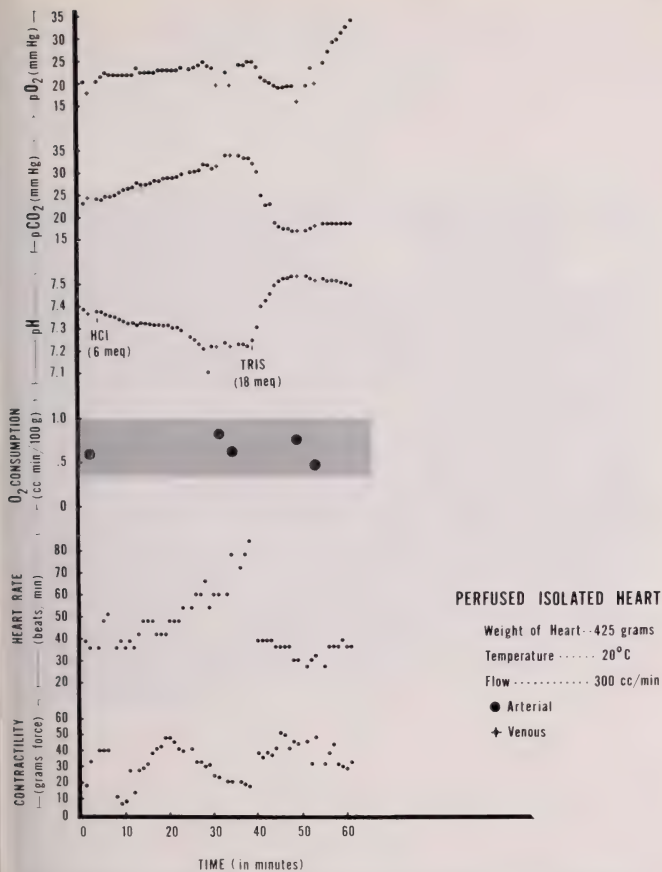


FIG. 9. Oxygen consumption of hypothermic isolated heart during perfusion with venous blood of varying pH.

7 to 8 and are less active at a lower pH. When fibrillating dog hearts, in which a changing heart rate or contractility could not influence oxygen consumption, were perfused, no increased oxygen consumption was found which could have been ascribed to an increase in dissociation of oxygen. It is probable that the

metabolic needs of the hypothermic heart are satisfied by an adequate coronary flow of venous blood (11) and that even an additional supply of dissociated oxygen could not be measured as increased consumption. This explanation still had to be tested by perfusing an entire animal with venous blood.

A very simple extracorporeal circuit (Fig. 10) can be set up for this purpose. Venous blood from the right heart drains through a jugular vein into an elastic chamber (12), is cooled in a heat exchanger (13) and pumped by a ventricle

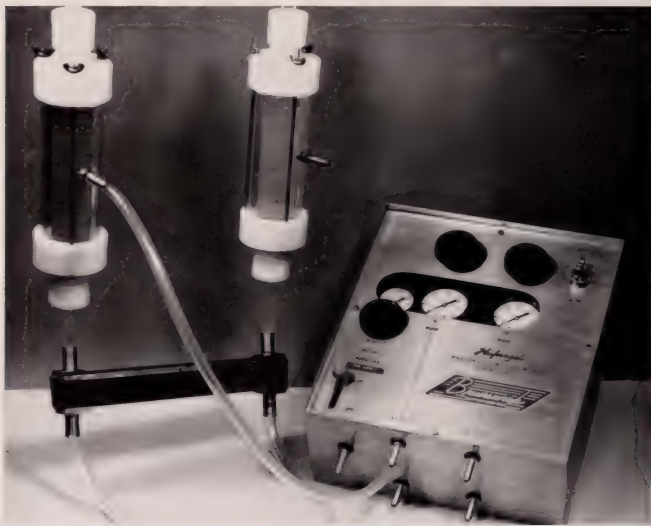


FIG. 10. Extracorporeal circuit for hypothermic perfusion with venous blood. Thin-walled "snitch" open to atmospheric pressure (right), heat exchanger (center) and ventricle pump with control unit (left).

pump (14) into a femoral artery. This is a small, closed, and atraumatic circuit which can be primed with 600 ml of Ringer's solution. No blood leveling devices are needed because if the pump exceeds the inflow, the elastic chamber collapses and prevents further inflow. The animals were anesthetized with a short-acting barbiturate and with fluothane and ventilated for one hour with oxygen, or with oxygen and increasing concentrations of carbon dioxide. During that time they were given 30 mg/kg of quinidine by slow i.v. drip to prevent ventricular fibrillation (4). Then partial cardiopulmonary bypass from one jugular vein to a femoral artery and blood stream cooling was started. When 15°C esophageal temp. was reached, the heart was made to fibrillate by

transthoracic electric shock and the respirator was stopped. However, veno-arterial perfusion continued at the same rate and the decline in the oxygen content of the blood served as an indicator of oxygen consumption. There was

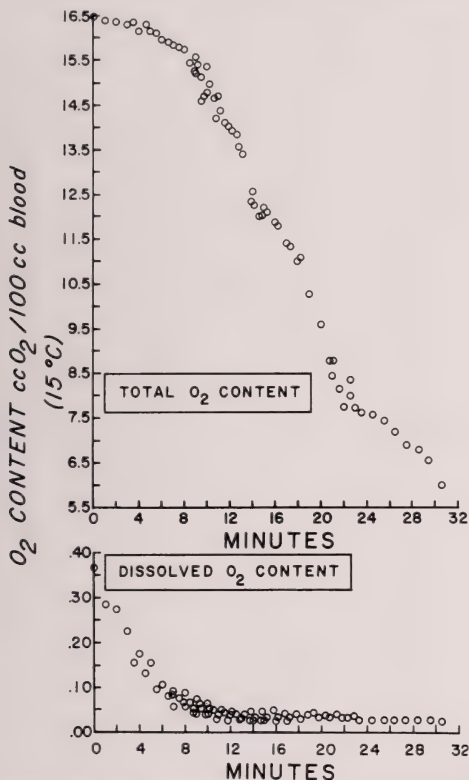


FIG. 11. Decline of total and of dissolved oxygen content during hypothermic perfusion with venous blood after induced cardiac arrest.

a very rapid decline in the oxygen content of the venous blood perfusing the body, and in the first five minutes the dissolved oxygen was used up because it was immediately available to the cells (Fig. 11). Because of the low oxygen tension of the venous blood this amount was small and from then on the body drew on the oxygen bound to hemoglobin and myoglobin until all available

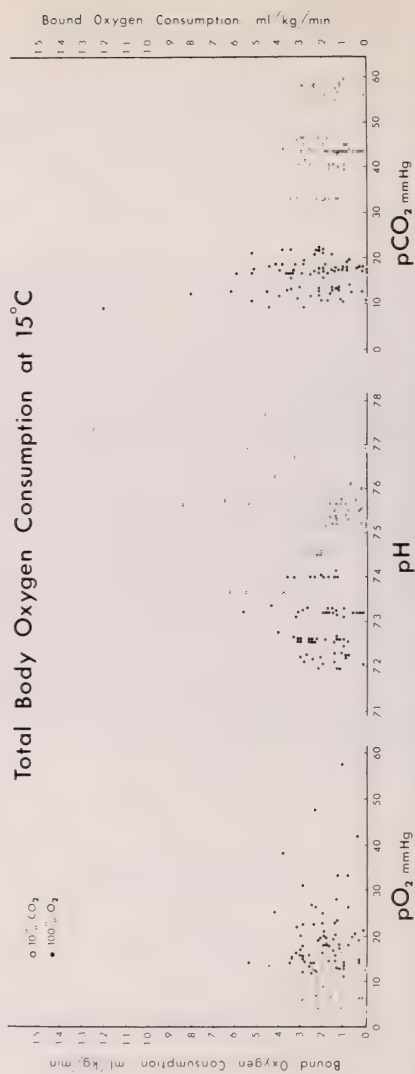


Fig. 12. Lack of correlation between "bound" oxygen consumption and pO<sub>2</sub>, pH and pCO<sub>2</sub> during hypothermic perfusion with venous blood.



oxygen was used up. Without seeking refuge and solace in the possibility of statistical significance, one look at the random scatter of the great number of determinations convinces us that all correlations lack biological significance (Fig. 12). Thus, there is no relation between the acidity of the blood produced by high carbon dioxide tension and the oxygen consumption of the hypothermic hypoxemic animal. Does this mean that the gas laws of Barcroft and King of the temperature shift to the left, or of Bohr, Hasselbach and Krogh of the  $\text{CO}_2$  shift to the right, are incorrect? Of course not. It simply means that under the conditions of this particular experiment of hypothermic perfusion their effect is not detectable and that no clinical benefit can be expected from the appli-

### THE REGULATION OF RESPIRATION

#### X. EFFECTS OF CARBON DIOXIDE, SODIUM BICARBONATE AND SODIUM CARBONATE ON THE CAROTID AND FEMORAL FLOW OF BLOOD

Detlev W. Bronk and Robert Gesell

(*Am. Journal of Physiology*, 82: 170, 1927)

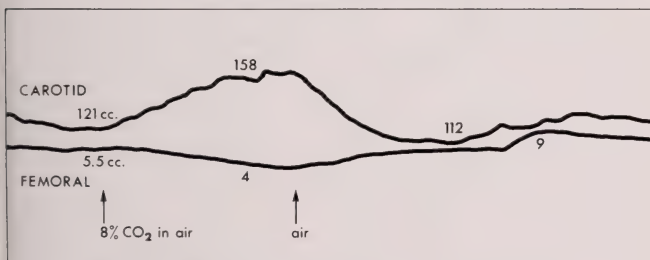


FIG. 13. The discovery of the carbon dioxide "master switch of life."

cation of this principle. But, does it also mean that it does not matter whether we use a carbon dioxide tension of 10 or 100 mm Hg? Of course not. The gas laws should certainly not be repealed, but we need additional legislation about the pharmacological effects of the various gases during hypothermic perfusion, particularly the effect of carbon dioxide.

In 1927 Bronk and Gesell (15) published a finding of such fundamental importance that a reproduction of one of their experiments may serve to inscribe it indelibly in our minds (Fig. 13). They made the original observation that an increase of carbon dioxide in the blood of a dog slowed down the blood flow in the muscles, but increased the circulation to the brain. P. F. Scholander has called this redistribution of the circulation which produces a heart and brain preparation, the master switch of life since it is the animal's ultimate defense against asphyxia (16). In a controlled study on fifty patients W. G. Lennox and E. L. Gibbs have shown in 1932 that man is no exception to this

biological law (17). When the nitrous oxide technique of measuring cerebral blood flow was used by Kety and Schmidt in 1945 (18), they found that the inhalation of 7% carbon dioxide doubled the cerebral blood flow.

The effect of carbon dioxide is particularly pronounced in hypothermia and

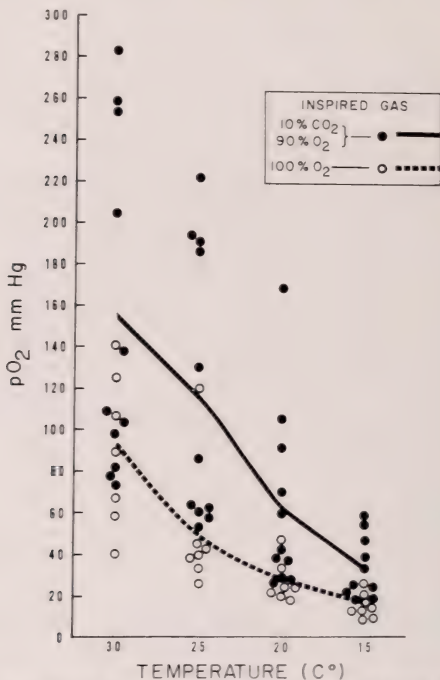


FIG. 14. Effect of carbon dioxide on venous  $pO_2$  during hypothermic perfusion with venous blood.

it has impressed investigators long before accurate methods of measurement were available. At the end of the last century Dubois induced hibernation in marmots by letting them breathe 12% oxygen and 45%  $CO_2$ , and wrote "l'acide carbonique en s'accumulant dans le sang, à l'état normal, provoque le sommeil d'abord, le réveil ensuite" (19). Whether his theory of  $CO_2$  narcosis of hibernation stood the test of time does not matter as much as his observations which we should have recorded better in our memory. The rat in the

closed fruit jar in Giaja's icebox was breathing a final mixture of 3% oxygen and 16%  $\text{CO}_2$  and survived  $8^\circ\text{C}$  (20). We have used the usually available mixture of 5%  $\text{CO}_2$  and 95% oxygen as the respiratory gas before and during deep hypothermia, and although the heart does not behave differently we have observed a better equilibration of temperature throughout the body as a sign of improved capillary flow. For deep hypothermia a higher ratio of  $\text{CO}_2$  to  $\text{O}_2$  may prove to be the gas mixture of choice. During total cardiopulmonary by-

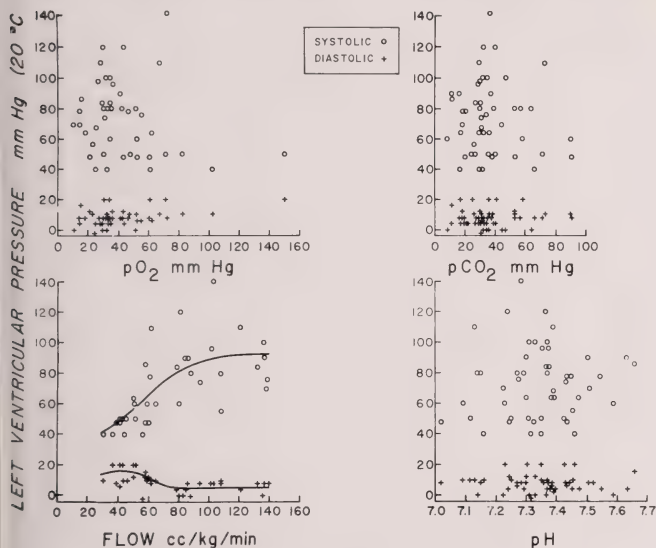


FIG. 15A

pass Peirce (21) equilibrated blood with 50% oxygen and 50%  $\text{CO}_2$  while the esophageal temperature was lowered to  $8^\circ\text{C}$ . At the end of the rewarming period of  $\text{pCO}_2$  of 350 mm Hg, a pH of 6.7 and nonrespiratory acids of 12 mEq/l were recorded. The moment the lungs came into play during partial cardiopulmonary bypass the  $\text{pCO}_2$  went down and the dog got up. This does not mean that the particular gas mixture in this important experiment should be applied clinically, but it indicates that different gas pressures may be required to sustain mammalian life at  $38^\circ$  and at  $8^\circ\text{C}$ . Before an answer to this question becomes available the danger sign of the skull and crossbones should be put on gas tanks containing such a toxic vasoconstrictor as pure oxygen. Deep hypothermia by perfusion abolishes the constancy of the "milieu interieur"

for a specific purpose and we mistake Claude Bernard's terminology for his spirit if we hold on with rigid scholasticism to one last vestige of a condition which does not exist any more.

We have undertaken a systematic study of a wide range of carbon dioxide tensions during hypothermic perfusion. As in the previous investigations on the carbon dioxide effect on oxygen consumption, right atrial blood was drained

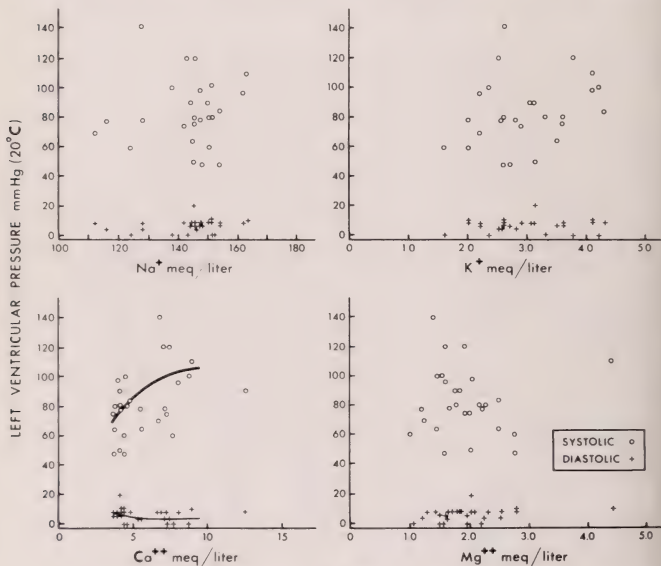


FIG. 15B

Fig. 15. Factors with (flow,  $\text{Ca}^{++}$ ) and without ( $\text{pO}_2$ ,  $\text{pCO}_2$ , pH,  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Mg}^{++}$ ) effect on left ventricular pressure during hypothermic perfusion with venous blood.

into an elastic chamber and heat exchanger and returned under pressure into a femoral artery. The closed extracorporeal circuit was filled with 600 cc of Ringer's solution. As could be expected, the venous blood of dogs breathing 10% carbon dioxide in oxygen had a higher oxygen tension than when 100% oxygen was used in the respirator (Fig. 14). There was considerable overlapping during moderate hypothermia, but since ventricular fibrillation was prevented by pretreatment with intravenous quinidine, the distinctly higher venous oxygen tensions of dogs breathing 10% carbon dioxide could also be shown during deep hypothermia.

Thus, the inhalation of 10%  $\text{CO}_2$  in oxygen accelerates cerebral circulation

to such an extent that the still well oxygenated blood in the superior caval vein may be adequate to cover the reduced oxygen consumption during hypothermic perfusion. We tested this assumption in venoarterial perfusions at 23°C esophageal temperature lasting three hours. During such a long period any

### HYPOTHERMIC PERFUSION WITH VENOUS BLOOD AT 23°C FOR 3 HOURS

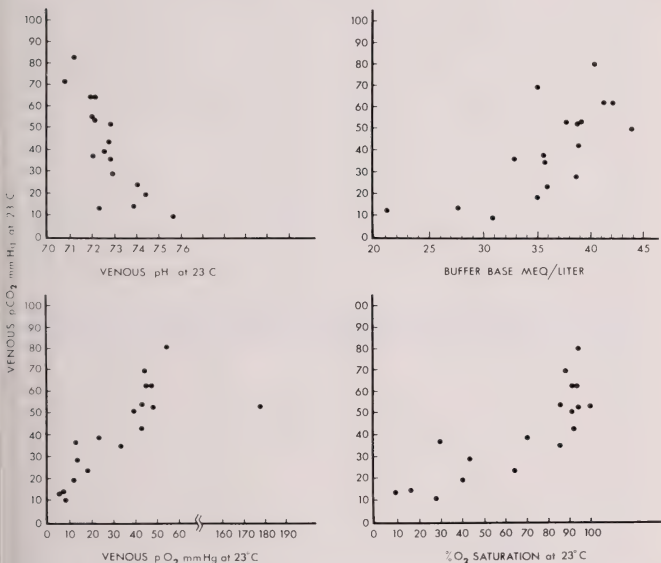


FIG. 16. Effect of carbon dioxide inhalation on venous pH, buffer base, pO<sub>2</sub>, and O<sub>2</sub> saturation during prolonged perfusion with venous blood at 23°C.

major violation of aerobic glycolysis due to prolonged lack of capillary perfusion would be unmasked as anoxic acidosis.

The esophageal temperature of 23°C was chosen because at that temperature the heart muscle develops the highest myocardial contractility (22) if about 100 cc·Kg/min of venous blood is shunted into a femoral artery. Under these conditions only the flow rate and calcium influence left ventricular pressure, whereas pO<sub>2</sub>, pCO<sub>2</sub>, pH, sodium, potassium, and magnesium concentrations have lost the importance they normally occupy (Fig. 15).

The pronounced and remarkably uniform effect of a one-hour long inhala-

tion of oxygen and of oxygen with increasing concentrations of carbon dioxide before hypothermic perfusion with venous blood becomes evident when we correlate the  $p\text{CO}_2$  with pH,  $p\text{O}_2$ , %  $\text{O}_2$  saturation and buffer base (Fig. 16). The severe hypocarbia due to hyperventilation with oxygen is accompanied by almost complete deoxygenation of venous blood and severe metabolic acidosis (23). If, however, the normal arterial  $p\text{CO}_2$  is doubled and raised to 80 mm Hg before venoarterial cooling, the venous oxygen tension remains high enough to achieve full venous oxygen saturation and to prevent a loss of buffer base. In fact, the venous  $p\text{O}_2$  is almost as high as under 3.5 atmospheres of oxygen pressure at normal temperature (24).

High tissue oxygen tension can be achieved by a high rate of capillary blood

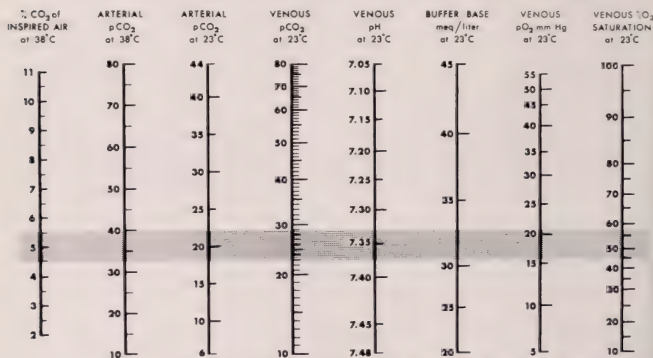


FIG. 17. Nomogram for the approximation of gas exchange during hypothermic perfusion with venous blood.

flow at normal atmospheric pressure, or at a low rate of capillary blood flow at increased atmospheric pressures. At the present state of knowledge it is simpler, safer and cheaper to speed up the turnover of oxygen for a prolonged period under the atmospheric pressure mammals have adjusted to in their evolution, than to reduce the turnover and to increase the gas tension to the short time limit of toxicity. The pleasure of observing the unusually rapid and full recovery of dogs after three hours of perfusion with cooled venous blood of high carbon dioxide content is enhanced by reading the words of August Krogh written half a century ago: "Even a relatively small decrease in the carbon dioxide content of the blood and tissues may produce the most strange and deleterious effects on the entire organism. It has been the important result of recent investigations that carbon dioxide can no longer be regarded as a mere waste product, for the organism only to get rid of; rather it must be accepted as one of the essential factors governing the processes of life" (25).

If one knows only the inhaled gas mixture at the start of the experiment



and follows the outlined procedure, one may get a fair approximation of the final venous oxygen saturation from a tentative nomogram (Fig. 17).

Unless new principles of propulsion or oxygenation of blood should be discovered, new advances in the field of extracorporeal corporation will come from the physiological laboratory and not from the machine shop. The present study on hypothermic perfusion with venous blood shows that the induction of hypercarbia permits even the deletion of an oxygenator. Thus, by relying on biological instead of mechanical engineering, a combination of reduced total oxygen consumption, lowered blood viscosity and peripheral resistance and of increased myocardial contractility can be produced which may prove to be beneficial in prolonged assistance to a failing circulation.

We should always keep in mind that the concept which has made prolonged extracorporeal circulation as well as hypothermic perfusion possible is based upon the attempt to overcome tissue anoxia by mechanical devices. As long as this principle is a fruitful one we should make full use of it in the operating room as well as in the laboratory. But we should abandon this concept immediately the moment a better one appears in the parade of permanent change. The faster the progress, the shorter the life span of a working hypothesis. We are not dedicated to the preservation of machines, techniques, or concepts—but to the progress of science itself.

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# Hemodynamic Considerations in Complete Heart Block

PHILIP SAMET, M.D., AND WILLIAM H. BERNSTEIN, M.D.\*

Present day therapy for patients with complete heart block and slow ventricular rates has emphasized the utilization of implantable electronic pacemakers, especially in individuals with Stokes-Adams episodes. Establishment of a firm theoretical and clinical basis for such therapy requires consideration of at least three questions: 1) What are the hemodynamic abnormalities in complete heart block? 2) What is the effect of variations in ventricular rate per se upon these hemodynamic abnormalities? 3) What is the significance of the atrial contribution to ventricular function?

## HEMODYNAMIC DATA IN COMPLETE HEART BLOCK

The hemodynamic aberrations associated with presumably acquired complete heart block had been studied in thirteen subjects (1, 2). Levinson *et al.* (1) performed right heart catheterization in five subjects (ages 52 to 78) with complete heart block. None had ever been in heart failure. The pertinent findings may be summarized as follows. Right atrial systolic peak pressures were elevated as were right ventricular and pulmonary artery systolic pressures. Increased pulse pressure was observed in the pulmonary and systemic arterial trees. The cardiac output was decreased but the stroke volume increased because of the slow ventricular rates. The highest atrial peak pressures were present when atrial contraction occurred just after closure of the tricuspid valve; atrial systole just prior to ventricular systole also resulted in elevated atrial peak pressures. Stack *et al.* (2) made comparable observations in eight subjects similarly studied. In four elderly subjects (ages 53-77) who had never been in heart failure and who sought medical aid because of Stokes-Adams episodes, elevated right heart pressures were observed together with reduced cardiac output, increased stroke volume, and increased arteriovenous oxygen differences. Right ventricular end-diastolic pressures were generally normal. In a fifth subject never in failure, aged 39, cardiovascular dynamics were identical but the stroke volume was sufficiently increased to result in a normal cardiac output despite the slow heart rate. The presence of congestive heart failure in three subjects (ages 62-72) resulted in accentuation of the cardiovascular abnormalities and marked decrease in cardiac output. Whether these abnormalities are caused by the slow ventricular rate per se or also by the associated heart disease was not evident from the above observations. The increased arteriovenous oxygen differences in these subjects suggest that the heart disease per se plays a role in the diminution in cardiac output.

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Further data have been contributed by other recent studies (3, 4). Samet *et al.* (3) observed that indicator dilution cardiac indices were less than 2 L./Min.  $M^2$  in nine of seventeen patients; in seven subjects the cardiac index varied from 2.0–2.7 L. Min.  $M^2$ . In another subject the cardiac index was 3.69 during isuprel infusion. Pressure data were similar to those reported by Stack and Levinson; normal right ventricular end-diastolic pressures were noted. Escher *et al.* (4) have also reported decreased cardiac indices in sixteen such patients, despite a rise in stroke volume. Arteriovenous oxygen differences were elevated. The above-mentioned studies were obtained by standard right heart catheterization. Data obtained by transeptal left heart catheterization

## M. Ros. Complete Heart Block.

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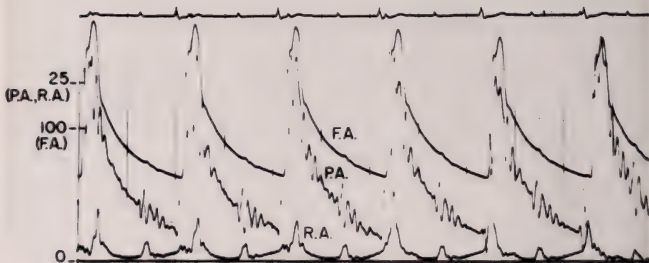


FIG. 1. Femoral and pulmonary artery and right atrial pressure curves in a patient with complete heart block and slow idioventricular rhythm. Atrial contraction against a closed tricuspid valve produces a large "a" wave in contrast to the normal "a" wave when the P wave precedes the QRS complex by at least a normal interval.

in a 40 year old woman have revealed changes in left atrial and ventricular pressures similar to those described for the right heart chambers; cardiac index was 2.05 L. Min.  $M^2$  and the left ventricular and systemic arterial pulse pressures were increased (5). Left ventricular end-diastolic pressure was normal. The temporal relationship between the P waves of atrial depolarization and the QRS complex of ventricular activation profoundly influences the form and level of the intracardiac pressure curves in patients with complete heart block (Figs. 1, 2). Atrial contraction against a closed mitral or tricuspid valve (closed as a result of ventricular contraction when the P wave is buried within, or follows the QRS complex) results in a higher atrial pressure with a giant cannon "a" wave compared to the atrial pressure curve following a P wave preceding a QRS complex by at least a normal "P-R" interval. This is true in both right atrial (Fig. 1) and left atrial curves (Fig. 2). Other hemodynamic

consequences of asynchronous atrioventricular activity will be discussed below. Judge *et al.* (5a) have recently added further data in this field. Fourteen subjects (ages 26–77) years) were studied after implantation of a left ventricular variable rate pacemaker. Exercise in four patients caused a significant increase in cardiac output. Fourteen subjects (ages 26–77 years) were studied after implantation of a left ventricular variable rate pacemaker. Exercise in four patients caused a significant increase in cardiac output. The average resting cardiac output in twelve subjects was 4.1 L./Min.; unfortunately neither the index data nor body surface area data were published. Norepinephrine infusion in five patients also resulted in a consistent rise in output at a constant ventricular rate, from 3.27 L./Min. to 4.55 L./Min.

Benchimol *et al.* (5b) studied the hemodynamic consequences of complete heart block in a 45 year old male. The control cardiac index was markedly depressed, 1.46 L./Min.  $M^2$  at a rate of 35. After insertion of a variable rate pacemaker, the index rose to 2.65 at a rate of 72. Exercise under the latter condi-

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#### Effect of Sequential Atrial Activity

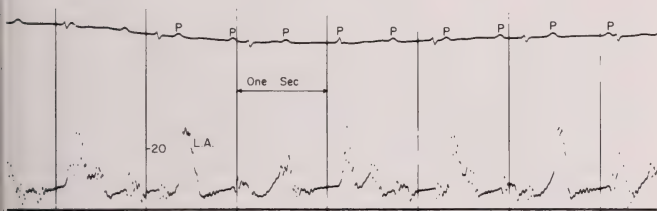


FIG. 2. Left atrial pressure curve in another patient with complete heart block. Giant "a" waves are again visible when left atrial contraction occurs against a closed mitral valve.

tions failed to increase the cardiac index; nitroglycerine sublingually in a dose of 1.150 Gr. also failed to alter flow during the implanted unit pacing. Marshall has also recently observed a low cardiac output (2.30 L./Min.) in eight patients (ages 63–78) with complete heart block and ventricular rates of 34–42/Min. (5c).

The hemodynamics of congenital complete heart block have recently been reviewed (6). These observations are of considerable interest since they probably represent the effects of a relatively slow ventricular rate per se without the superimposition of the associated myocardial disease as is generally true in acquired complete heart block. The heart rate ranges from 40–80 per minute. In spite of the bradycardia the cardiac output is usually normal because of the elevated stroke volume (7, 8); the latter is of a greater degree than that usually seen in acquired complete heart block. It is also possible that the more normal cardiac output in congenital heart block is at least associated with a ventricular pacemaker located at the atrioventricular node or His bundle (with a normal QRS complex) as compared to the idioventricular focus with a

widened aberrant QRS complex more frequently found in acquired complete heart block. Right heart pressures are generally normal in congenital complete heart block; the right ventricular and pulmonary artery systolic pressures may on occasion show moderate elevation (7, 8). The systemic and pulmonary arterial pulse pressures are widened. Ventricular end-diastolic pressures are normal or minimally elevated. Exercise data in both acquired and congenital complete heart block are limited. Escher *et al.* (4) exercised four patients (with acquired block) with fixed ventricular rates. Cardiac index increased 475 ml.  $M.^2$  Min. for an increase in oxygen consumption of 87 ml. Min./ $M.^2$ . The variations in atrial pressures which accompany the accidental P-R intervals in acquired complete heart block are also characteristic of congenital heart block (6-8). Other exercise data were mentioned earlier (5a, 5b).

In summary therefore it is apparent that there are definite hemodynamic abnormalities evident in most patients with acquired complete heart block and slow ventricular rates. These findings are less readily evident in congenital complete heart block. It remains for future study and discussion to determine whether increases in rate per se may correct some or all of these changes.

#### EFFECT OF VARIATIONS IN VENTRICULAR RATE UPON HEMODYNAMIC DATA IN COMPLETE HEART BLOCK

Most of the data relative to this problem has been obtained in the course of right ventricular outflow tract pacing with a pacemaker electrode catheter utilizing a unipolar catheter (9), with a bipolar catheter with both electrodes near the tip (10), or with a second type of bipolar electrode catheter in which the catheter tip is placed in either pulmonary artery. The two electrodes on this latter catheter are 15 and 18 cm respectively proximal to the tip and are placed of course in the right ventricular outflow tract (3).

Escher *et al.* (4) have reported studies in sixteen patients. The cardiac index was generally below 2 L. Min.  $M.^2$  as determined by the Fick principle. Although the individual patient data have not been published in full detail, the information is available in graphic form (4). In all subjects the cardiac index rose as the heart rate was increased from the slow idioventricular rate to faster catheter paced ventricular rates. The increments in cardiac index ranged from 16 to 110 per cent. In most patients the cardiac output continued to rise with the increase in heart rate within the range tested. The maximum rate of increase occurred between the basic idioventricular rate and rates in the range of 60 to 75. In several subjects cardiac output was still increasing at the maximum rate tested, i.e., that between 100-120. Two subjects exhibited decrease in flow at rates of 105 and 140. It is essential to note that the heart rate at which the cardiac index is maximal varied from patient to patient. Since the increase in flow was proportionately less than the rise in rate, stroke volume fell as the rate rose. It is of interest that the sinus rate did not vary as the ventricular rate was altered. Respiratory rate remained unchanged as the heart rate was increased. The arteriovenous oxygen differences were widened during idioventricular rhythm but fell as the heart rate and cardiac index rose. I



some patients the oxygen consumption was subnormal at the idioventricular rate (down as low as 90 ml./Min.  $M.^2$ ) but rose with increases in heart rate. Overall therefore, more normal cardiac output and related parameters were observed as the ventricular rates increased. Samet *et al.* (3) have made comparable observations in seventeen subjects employing the indicator dilution technique with indocyanine green injected into the right heart and sampled from a systemic artery. Five patterns of response to increasing ventricular rate were noted (Fig. 3). In five patients the rise in cardiac index with increasing ventricular rates was minimal, less than 10 per cent. In six patients the output rose more than 10 per cent with rate increments. However a secondary fall in output was observed as the ventricular rate increased further,

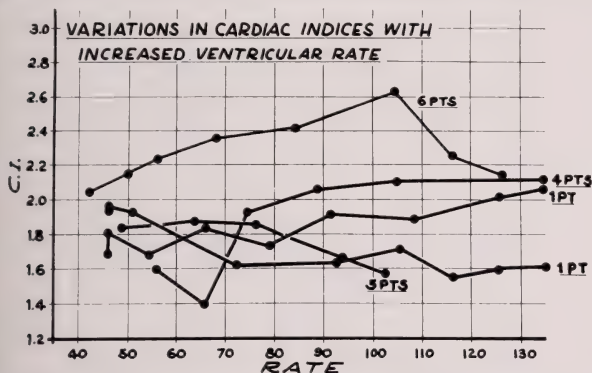


FIG. 3. Effect of variations in heart rate produced by a right ventricular outflow tract catheter electrode in 17 patients with complete heart block. In 11 of 17 patients, there is a significant increase in cardiac output as the heart rate is increased. See text for details.

resulting in a bell-shaped curve relating output and rate. Peak cardiac outputs were generally recorded at ventricular rates of 85–105 per minute but maximum outputs were also noted at lower ventricular rates. In one patient cardiac index continued to rise progressively as the rate was increased. In four patients the control cardiac index rose more than 10 per cent as the rate rose, to a higher level which however remained constant as further rate increments were induced. In one patient the maximum cardiac index was observed at the slowest ventricular rates of 46–51. At higher rates more than a 10 per cent fall in cardiac index was noted. Cardiac index remained constant at this lower level as the heart rate was further increased. Rate increments were therefore associated with output increments in eleven of the seventeen patients studied. Since the heart rate associated with a peak cardiac index varied from patient to patient, prediction of the optimal rate was not possible. In all five groups of patients stroke volume decreased with increases in heart rate.

Other investigators have published comparable data in smaller groups of patients. Wade and Bishop (11) have reported data in eight patients with complete heart block, quoting unpublished work by Segal and Hudson. The relationship between heart rate and cardiac output was explored at two levels of oxygen consumption, i.e., at rest and during exercise. The averaged resting control cardiac index was 2.1 L. Min. M<sup>2</sup>. Increase in the heart rate to the 70-90 range was accompanied by a rise in index to 2.8. Bevegard (12) has

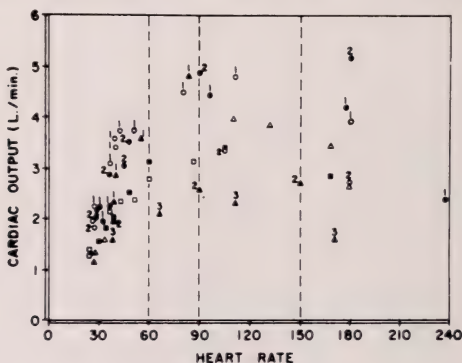


FIGURE 1

*Alterations in cardiac output associated with changes in ventricular rate. The different symbols in this and the following three figures indicate the six individual dogs. The numbers above the symbols indicate observations in the same dog on different occasions. Note the increase in cardiac output when the ventricular rate is increased to above 60 beats per minute.*

FIG. 4. Changes in cardiac output with changes in ventricular rate in the dog with complete heart block. See text for details. From Miller *et al.* (16).

studied two patients with complete heart block. The ventricles were paced by electrodes sutured into the myocardium. In the first patient the Fick cardiac outputs were 3.1, 3.7, 4.8, 6.1 and 5.5 L. Min. at heart rates of 22, 31, 57, 74 and 109 respectively. In the second patient the outputs were 3.6, 4.4, 6.0, 6.7 and 6.3 L. Min. at rates of 28, 55, 72, 91 and 99 respectively. Stroke volume fell as the heart rate rose. Lasry (13) found only minimal (less than 10 per cent) changes in output in a single subject as the heart rate rose from a control of 62 to a maximum rate of 104. Muller and Bellet (14) reported increases in output with rate increments in five patients as did Haupt *et al.* (15) in two patients.

Judge *et al.* (5a) analyzed the effect of increasing ventricular rate upon cardiac output in twelve subjects. Patients with initial outputs of 4.1 L./Min. exhibited little increase when the heart rate rose whereas those patients with control outputs of 3 L./Min. or less more often tended to increase their output significantly. At heart rates of 60, 75, 90 and 110 respectively, the cardiac outputs were 4.1, 4.3, 4.5 and 4.6 L./Min.; these increments represent 7%, 10% and 12% rises in output above control levels respectively.

Benchimol *et al.* (5b) studied one 45 year old male in complete heart block. At a rate of 35 the cardiac index was 1.46 L./Min./M.<sup>2</sup>. As the heart rate was

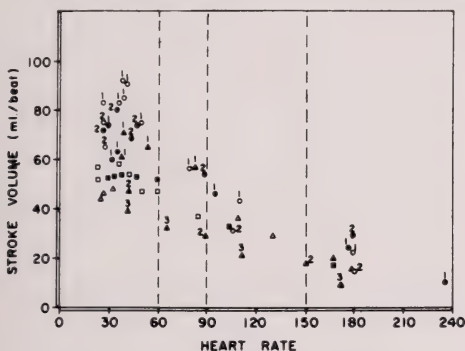


FIGURE 2

*Alterations in stroke volume associated with changes in ventricular rate. Note that the stroke volume remains relatively constant at rates below 60 and then decreases progressively as ventricular rate is increased.*

FIG. 5. Stroke volume changes with increments in heart rate. See text for details. From Miller *et al.* (16).

progressively increased, cardiac index reached a peak of 3.39 L./Min./M.<sup>2</sup> at a ventricular rate of 76. A further heart rate increment to 100 per minute did not further alter cardiac index, 3.30 L./Min./M.<sup>2</sup> Marshall (5c) investigated the effect of increasing ventricular rate upon cardiac output in complete heart block by catheter electrode right ventricular outflow tract pacing in 8 subjects with complete heart block. Control output of 2.30 L./Min. at rates of 34-42 rose to 3.63 L./Min. at rates of 60-80 Min. At faster rates, the output tended to fall slightly.

Miller *et al.* (16) have produced surgical complete heart block in the dog and analyzed the relationship between heart rate and cardiac output. The former was controlled by a right ventricular outflow tract catheter. Increasing the ventricular rate from the slower idioventricular rate to rates of 60 resulted in

a progressive rise in output. Heart rates between 60–90 resulted in modest output increments. Cardiac output remained fixed between rates of 90–150; above 150, cardiac output fell with increasing rates (Fig. 4). Stroke volume remained constant at rates below 60, but progressively decreased at higher rates (Fig. 5). Right atrial mean and right ventricular end-diastolic pressures were elevated at both extremes of the ventricular rates (Figs. 6, 7).

Escher *et al.* (4) have reported the effect of increasing the ventricular rate in sixteen patients via a right ventricular outflow tract catheter upon pulmonary

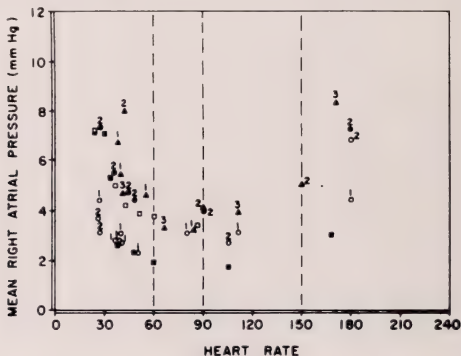


FIGURE 3

*Alterations in the mean right atrial pressure associated with changes in ventricular rate. Note the elevated mean right atrial pressures at both high and low ventricular rates with lower pressures at rates of 60 to 150/min.*

FIG. 6. Changes in right atrial pressure with rate changes. From Miller *et al.* (16).

artery systolic pressure. At the lower heart rates systolic pressure was often elevated, up to 85 mm Hg. As the heart rate and cardiac output rose during pacing, systolic pulmonary artery pressure fell. In a small group with normal control pulmonary artery systolic pressures, the latter rose with increments in heart rate. Mean pressure changed little while diastolic pressure rose as the rate and output increased. Pulmonary artery pulse pressure thus narrowed with rate increments. Similar changes were observed in systemic arterial pressures. Comparable data were found by Samet *et al.* in a group of seventeen patients (3).

Three studies (11, 12, 15) are available in man in which the effect of varying ventricular rate during similar exercise loads was analyzed. In all three exer-

cise studies cardiac output rose at the higher ventricular rates much as most studies at rest have demonstrated.

In summary, these observations during idioventricular pacing point to the importance of variation in heart rate for the therapy of complete heart block. Recognition of this fact has led to the development of variable rate idioventricular pacemakers. (15, 17-20). These do not however permit synchronization of atrial and ventricular activity. The importance of the latter factor will be analyzed next.

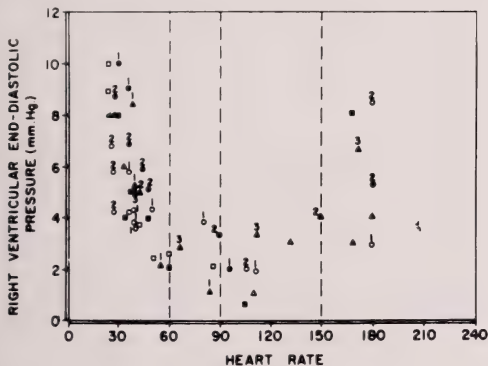


FIGURE 4

*Alterations in right ventricular end-diastolic pressure with changes in ventricular rate. Note similarity between the right ventricular end-diastolic pressure and the mean right atrial pressure (fig. 3).*

Fig. 7. Changes in right ventricular end-diastolic pressure with rate changes. Figs. 4-7 from same series of dogs. From Miller *et al.* (16).

#### IMPORTANCE OF ATRIAL SYSTOLE FOR VENTRICULAR FUNCTION

The above discussion has demonstrated that there are clearcut hemodynamic abnormalities in complete heart block and that at least some of these aberrations may be diminished by increasing the ventricular rate. Can synchronization of atrial and ventricular activity be of further aid? Animal (21-29) and clinical (5, 30-35) studies strongly suggest an answer in the affirmative. Burchell has recently reviewed the importance of the atrial transport function (33), stressing that interest in atrial function has redeveloped of late because of the newly available direct current conversion technique for atrial fibrillation, because of newly designed electronic pacemakers for the treatment of

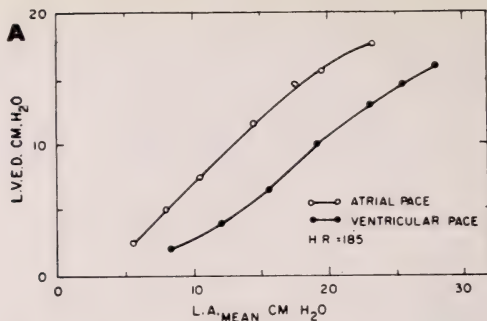


FIG. 4. A, LVED = left ventricular end diastolic pressure. L. A. mean = mean left atrial pressure. The open circles show the relation between left ventricular end diastolic pressure and mean atrial pressure during atrial pacing and the solid circles show the relation between these two variables during ventricular pacing at the same rate. B, the relation between cardiac output in liters per minute and mean atrial pressure in cm. H<sub>2</sub>O during atrial pacing is shown by the open circles and during ventricular pacing by the closed circles.

FIG. 8. Relationship between left atrial mean and left ventricular end-diastolic pressure, during atrial and ventricular pacing. See text for details. From Mitchell *et al.* (22).

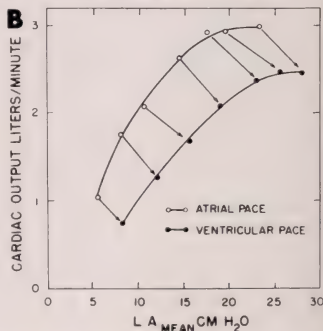


FIG. 9. Relation between left atrial mean pressure and cardiac output during atrial and ventricular pacing. Note the larger output during atrial pacing. From Mitchell *et al.* (22).

complete heart block and because of experimental data relative to the physiological importance of atrial function for ventricular performance.

Sarnoff and co-workers (21, 23, 26, 28) have demonstrated the importance of atrial systole for maintenance of cardiac output, closure of the atrio-ventricu-



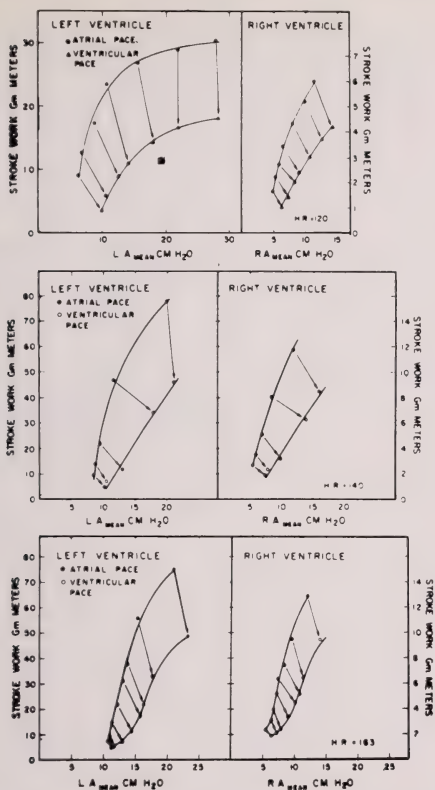


FIG. 2.—Simultaneous left and right ventricular function curves (V.F.C.LA) and V.F.C.RA) from three experiments. In each instance the arrows indicate the change in mean atrial pressure and ventricular stroke work that took place when the stimulus was changed from atrium to ventricle. H.R.=heart rate. Bilateral cervical vagotomy and left stellate ganglionectomy.

FIG. 10. Right and left ventricular function curves relative to atrial and ventricular pacing. Ventricular stroke work is consistently greater during atrial pacing. From Gilmore *et al.* (27).

lar valve and for prevention of insufficiency at the latter valve. The significance of the exact temporal relationship between atrial and ventricular activity has been examined. At the same left atrial mean pressure, left ventricular end-diastolic pressure and cardiac output are both greater during atrial as compared to ventricular pacing (Figs. 8, 9). Left and right ventricular stroke work (Fig. 10) are also larger during atrial pacing. These differences during atrial pacing are attributed to the atrial "kick" provided by atrial systole. Atrial systole at a normal As-Vs (or P-R) interval of 80 milliseconds, provides maximal stroke work and stroke volume and aortic mean pressures, while

**TABLE 1.** *Hemodynamic effects of lengthening and shortening the interval between atrial systole and ventricular systole*

	Control As-Vs 75-120 msec.	As-Vs >200 msec.	Control As-Vs 75-120 msec.	As-Vs <50 msec.
MLAP, cm H <sub>2</sub> O	8.3±0.53	11.1±0.49	9.3±0.62	11.8±0.68
MAP, mm Hg	82.6±1.71	71.1±1.51	76.2±2.40	67.5±2.28
Aortic flow, ml/min	2215±96.6	1888±89.1	2256±116.8	1962±105.6
MLAP- LVEDP, cm H <sub>2</sub> O	-4.2±0.42	0.0±0.36	-1.8±0.45	+1.9±0.48

Values are mean and standard error. As-Vs = atrial systole-ventricular systole interval in msec; MLAP = mean left atrial pressure; MAP = mean aortic pressure; MLAP-LVEDP = mean left atrial pressure minus left ventricular end-diastolic pressure. Prolonged As-Vs interval = 43 observations in 11 animals. Shortened As-Vs interval = 29 observations in 9 animals. Average ventricular rate for the group in which the As-Vs interval was prolonged was 146, and for the group where the As-Vs interval was shortened was 148.

FIG. 11. Effect of P-R interval on cardiovascular dynamics in the dog. Increase or decrease of the P-R interval lowers aortic pressure and cardiac output. From Skinner *et al.* (28).

mean left atrial pressures are minimal (Figs. 11, 12). Increase or decrease of the P-R intervals to abnormal levels results in a decrease in output and peripheral arterial pressure and a rise in mean left atrial pressure. Such abnormal P-R intervals are also associated with mitral insufficiency (Fig. 13). Stephenson and Brockman (36) recorded aortic pressure in five dogs with surgically produced complete heart block, during idioventricular and P wave synchronous pacing. The average mean aortic pressure was 149 mm Hg during atrial pacing, and 132 mm Hg during ventricular pacing. Brockman (29) has analyzed atrial and ventricular pressure curves in dogs with surgically produced complete heart block. He noted that atrial contraction propagated a pressure wave toward the ventricle. The latter pressure wave produced a nega-

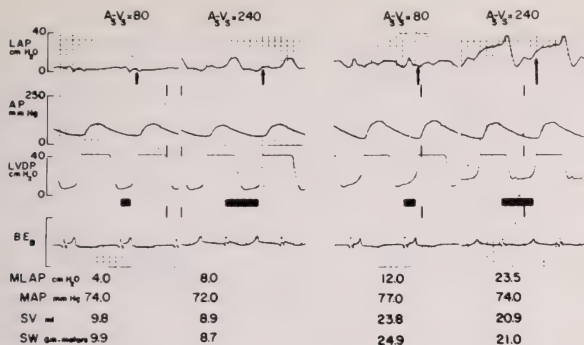


FIG. 3. Effect of prolonging the A-Vs interval (msec) at two levels of stroke volume. Ventricular rate constant at 134/min. Mean aortic pressure and stroke volume held essentially constant before and after each intervention. Black bars represent A-Vs

interval. Vertical arrows point to atrial pressure during isovolum ventricular systole. Abbreviations as in Figs. 1 and 2. Paper speed 100 mm/sec.

FIG. 12. Effect of P-R interval on cardiovascular dynamics in the dog. From Skinner *et al.* (28).

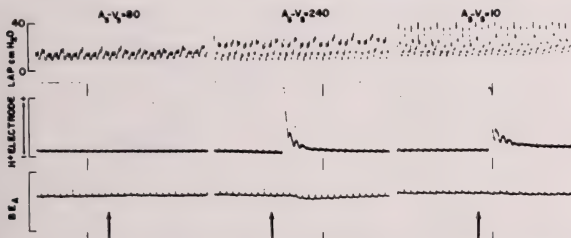


FIG. 4. Mitral regurgitation during improper timing of atrial systole determined by platinum electrode (H<sup>+</sup> electrode) technique. Left panel is proper timing of atrial systole (A-Vs 80 msec); middle panel = prolonged A-Vs interval (240 msec); right panel = shortened A-Vs interval (10 msec). Arrows mark onset of ascorbic

acid injection into the left ventricle. No deflection noted with proper timing, prominent deflections occurred with prolonged and shortened the A-Vs interval. Paper speed 10 mm/sec ventricular rate 134/min.

FIG. 13. Effect of P-R interval on production of mitral insufficiency. Left atrial sampling after left ventricular injection of ascorbic acid. Shortening or lengthening of the normal P-R interval results in early left atrial appearance of ascorbic acid, i.e., mitral insufficiency. From Skinner *et al.* (28).

tive difference in atrio-ventricular pressure, i.e., with atrial pressure less than ventricular, with subsequent closure of the atrio-ventricular valve. The negative difference in atrio-ventricular pressure was greatest at a normal "P-R" interval of about 0.15 sec, and fell rapidly as the accidental "P-R" interval decreased or increased from the 0.15 sec level in these animals with complete heart block (Fig. 14). In addition, Brockman noted that atrial systole produced a rise in ventricular end-diastolic pressure and fiber length with a re-

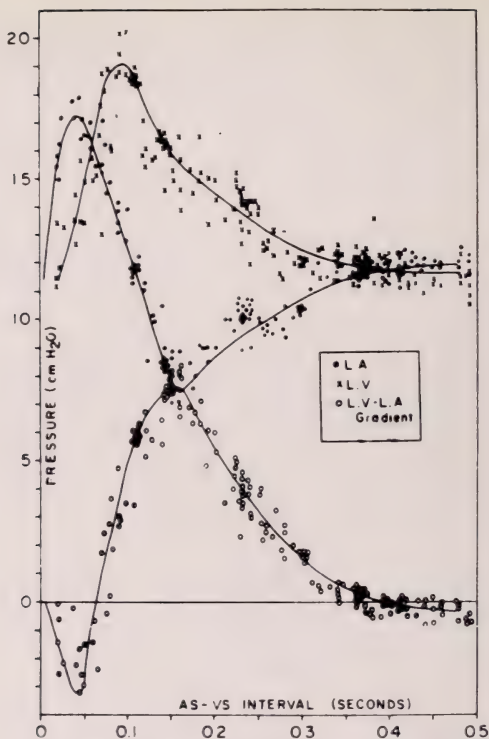


FIG. 3. Effect of atrial contraction on end-diastolic left atrial and left ventricular pressure over a wide range of AS-VS intervals. Lower curve is the differential pressure between atrium and ventricle at end-diastole. Portion of the curve below the line indicating zero differential pressure is the positive difference in A-V pressure and the portion of the curve above this line is the negative difference in A-V pressure. Curve constructed from the analysis of a large number of ventricular beats from a dog with complete A-V block.

Fig. 14 Effect of accidental "P-R" interval upon atrio-ventricular pressure differences in dogs with surgically produced complete heart block. See text for detail. From Brockman (29).

sulting augmentation in ventricular response. At the most ideal "P-R" incidental interval of 0.15 sec, aortic pressure, stroke volume, and left ventricular stroke work were maximal (Fig. 15). Sarnoff has also concluded from experimental studies that atrial systole may play a role in closure of the atrio-ven-

tricular valve (26). Grant *et al.* (35) have come to a similar view after right heart catheterization in man with complete heart block. Atrial systole elevated ventricular pressure by 1–3 mm Hg, with development of a reverse or negative atrio-ventricular gradient and closure of the tricuspid valve (Fig. 16). These recent studies have reinforced the earlier conclusions of Gesell (24), Wiggers (25), and Joehim (37a) as to the importance of atrial systole.

Data leading to similar inferences are available via studies in man. Braun-

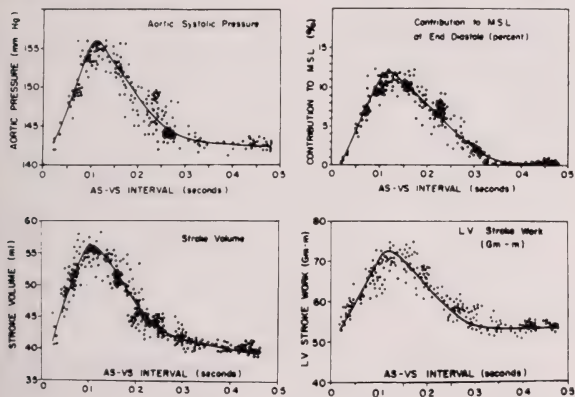


FIG. 4 Effective contribution of atrial contraction to aortic pressure, initial ventricular fiber length, stroke volume, and stroke work over a wide range of AS-VS intervals. Each curve was constructed from the analysis of a large number of ventricular beats in dogs having complete A-V block.

Fig. 15. Same group of experimental animals as in Fig. 14. Effect of P-R interval upon hemodynamic parameters. From Brockman (29).

wald and Frahm (30) investigated the relationship between mean left atrial pressure and left ventricular end-diastolic pressure in man. Transient atrio-ventricular dissociation occurred during left heart catheterization in a patient with aortic stenosis. Four pressure pulses of the left ventricle and brachial artery were illustrated together with the electrocardiogram (Fig. 17). In the first beat the P-R interval was 0.19 sec; in the second beat the P-R interval was 0.13 sec; in the third beat the P wave was hidden within the QRS complex; in the fourth beat the P wave followed the QRS complex and the R-P interval was 0.13 sec. The respective left ventricular end-diastolic pressures were 30, 21, 15 and 12 mm Hg suggesting a progressive decrease in left ven-

tricular filling as the temporal relationship of atrial and ventricular contraction was altered. Further evidence for such an interpretation of the effect of changing the sequence of atrial and ventricular activity lay in the progressive fall of systolic pressure in the brachial artery during these four beats—142, 139, 128 and 120 mm Hg respectively. The corresponding left ventricular systole pressures were 233, 225, 202 and 194. The duration of left ventricular

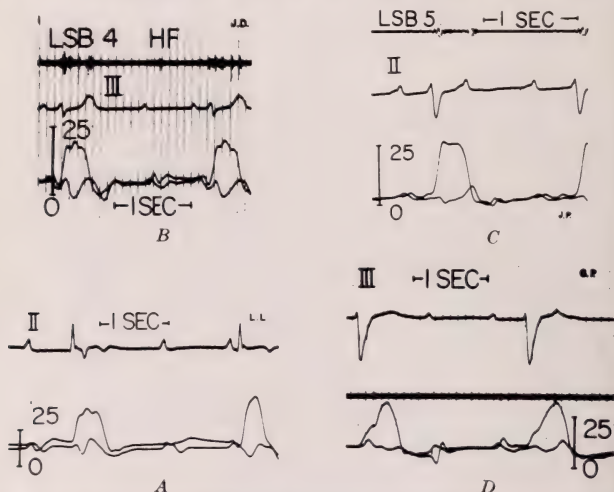


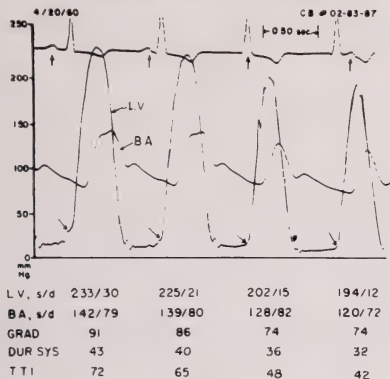
FIG. 2-5. Records of right heart catheterization showing the effect of isolated atrial contractions on atrial and ventricular pressures. Ventricular pressure is elevated by 1 to 3 mm. Hg at the end of the atrial contraction cycle, while atrial pressure has returned to its previous level, so that a reverse gradient has developed across the tricuspid valve. In Figure 2A the ventricular pressure curve has been separated slightly from the atrial record. LSB4, HF = high frequency phonocardiogram from the fourth intercostal space, left sternal border (400 to 2,000 c.p.s.). LSB5 = phonocardiogram from the fifth intercostal space, left sternal border (low frequency, 40 to 200 c.p.s.).

FIG. 16. Right atrial and ventricular pressure curve in patient with complete heart block. Transient reversal of atrioventricular pressure gradient, with atrial pressure less than ventricular; atrial systole may thus play a role in closure of the tricuspid valve. From Grant *et al.* (35).

systole, the peak left ventricular-brachial artery gradient and the tension time index in mm Hg-sec also underwent progressive decreases. Samet *et al.* (5, 31) have published striking examples of the effect of changes in P-QRS temporal relationships upon right and left heart and systemic arterial pressures during idioventricular rhythm and pacer rhythm in patients in complete heart block. Normal "P R" intervals are associated with higher systemic arterial curves and more normal right atrial pressures than when the P wave is buried within or follows the QRS complex (Fig. 18). The cyclic variations in femoral artery and right heart curves make a striking picture (Figs. 19, 20)



Left ventricular end-diastolic pressure is considerably higher during sinus rhythm than during atrial fibrillation, in a patient with aortic valve disease, despite the more rapid rate during the atrial arrhythmia (Figs. 21, 22). The absence of coordinated atrial and ventricular activity during a series of ven-



**Figure 8**

*Simultaneously recorded left ventricular (L.V.) and brachial artery (B.A.) pressure pulses obtained from a patient with aortic stenosis and atrioventricular dissociation. The vertical arrows near the top point to the P waves on the electrocardiogram; the oblique arrows near the bottom of the tracing indicate the left ventricular end-diastolic pressure. The figures below each beat indicate the pertinent hemodynamic measurements. S d = systolic diastolic pressures in mm. Hg. GRAD. = pressure gradient in mm. Hg between the peak left ventricular and peak brachial artery pressures, DUR. SYST. = Duration of systole in seconds, between onset of ventricular contraction and aortic valve closure, T.T.I. = Tension-time index in mm. Hg-sec.<sup>16</sup>*

FIG. 17. Hemodynamic effect of transient atrio-ventricular dissociation in patient with aortic stenosis. See text for details. From Braunwald and Frahm (30).

ricular premature beats is also reflected in the lower left ventricular end-diastolic pressure during the aberrant beats (Fig. 23). Escher *et al.* (4) have recently published right heart and systemic arterial pressure curves showing similar variations in response to variations in the P-QRS temporal relationships. These observations demonstrate that properly timed atrial systole permits an increase in ventricular contractile force without elevation of atrial

mean pressure by the mechanism of the "atrial kick" contribution to ventricular end-diastolic pressure.

Judge *et al.* (5a) have also shown the importance of atrial systole in cardiovascular dynamics, in two patients with complete heart block treated with implantation of left ventricular variable rate pacemakers. In both subjects the pacemaker rate was adjusted with an external control unit until the ven-

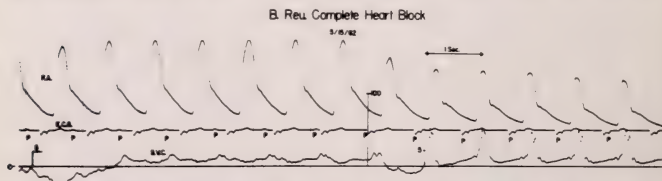


FIG. 18. Electrocardiogram and femoral artery and superior vena cava curves. Temporal changes in the P-QRS intervals are associated with femoral artery pressure level changes as well as ventricularization of the superior vena cava curve highly suggestive of tricuspid insufficiency. Catheter electrode right ventricular pacing in patient with complete heart block.

### E. Ket. Intermittent Complete Heart Block

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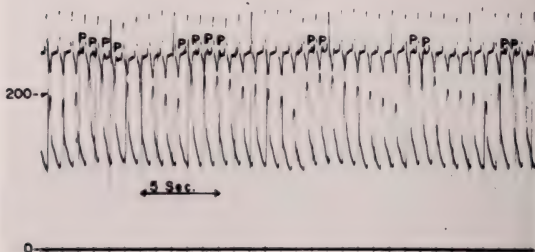


FIG. 19. Complete heart block patient; implanted idioventricular pacemaker. Note marked variations in systemic arterial pressure levels with variations in the P-QRS time relations.

tricular and atrial rates were identical with a "P-R" interval of about 0.20 sec. Stroke volume and cardiac output were significantly higher when atrial contraction preceded ventricular activity than when the two chambers contracted independently but at the same ventricular rate. In one patient at a rate of 60, cardiac output was 6.0 L. Min. during coordinated activity but only 4.8 when atrial and ventricular activity were non-coordinated. In the second patient at a ventricular rate of 102, the cardiac output rose from 5.1 to 6.1 L. Min. when coordination was established. The duration of ventricular injection increased when atrial activity preceded ventricular contraction by

0.20 sec. Marshall (5c) has also reported marked systemic arterial blood pressure variations during catheter electrode idioventricular pacing in patients with complete heart block. These pressure fluctuations amounted to as much as 48 mm Hg.

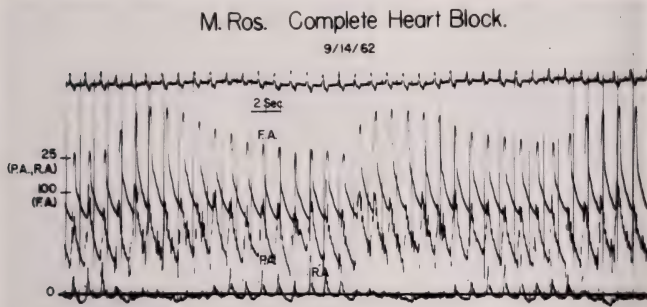


FIG. 20. Pulmonary and femoral artery and right atrial pressure curves during right ventricular catheter pacing in a patient with complete heart block. Note marked repetitive variation in arterial pressure levels and appearance of intermittent giant "a" waves in right atrial curve.

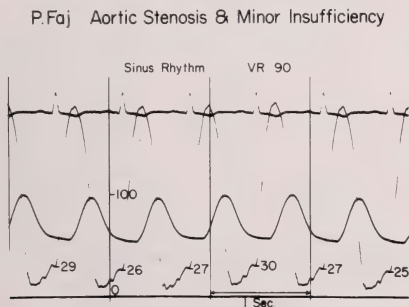


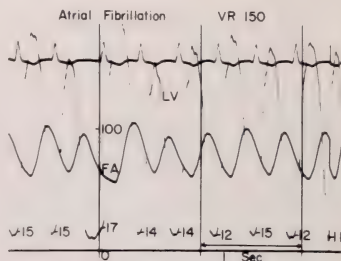
FIG. 21. Patient with aortic valve disease during sinus rhythm. Left ventricular diastolic pressure varies from 25–30 mm.Hg.

The effect of the atrial systolic contribution to ventricular filling and cardiac output has also been studied. The work of Sarnoff *et al.* has been referred to previously (21–23, 26–28). Stephenson and Brockman (36) have determined the stroke volume in five dogs with complete heart block during atrial and during ventricular pacing at the same ventricular rate. The stroke volume during

the former type of pacing was 13.0 ml compared to 11.4 ml during idioventricular pacing. Sellers *et al.* found a 15 per cent increment in cardiac output when atrial systole preceded ventricular activity (37). Mitchell *et al.* reported a 19 per cent increment in output under the same conditions (38). Comparable data are available in man. Escher *et al.* (4) studied four patients at rest during

### P.Faj Aortic Stenosis & Minor Insufficiency

FIG. 22. Same patient as in Fig. 21. During atrial fibrillation, without the contribution of atrial systole, left ventricular end-diastolic pressure falls to levels of 11-17.



### P.Faj Aortic Stenosis & Minor Insufficiency

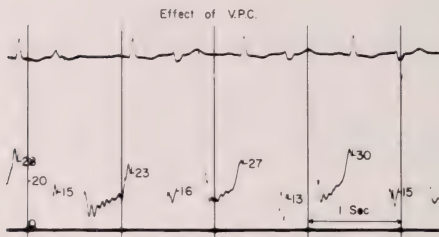


FIG. 23. Same patient as in Fig. 21-22. Bigeminal rhythm, with ventricular premature beats alternating with sinus conducted beats. Note the higher left ventricular end-diastolic pressure during sinus rhythm. Systolic left ventricular systolic peaks are off the top of the paper. The contribution of atrial systole to left ventricular end-diastolic pressure is evident.

atrial and ventricular pacing at similar heart rates. Cardiac index averaged 20% greater during atrial pacing. Nathan *et al.* (34) have performed comparison of cardiac index during synchronous atrio-ventricular pacing and during idioventricular pacing in four subjects. At least two months elapsed between the first (idioventricular) and second (synchronous) study in the same patient. Heart rates were similar but not necessarily identical during the two studies. In one patient cardiac index was similar during both pacing tech-

niques. In the other three, the cardiac index averaged about 18 per cent more during synchronous atrial than during idioventricular pacing. In another, as yet unpublished study from the present authors' laboratory, the effect of atrial and ventricular pacing by a bipolar electrode catheter placed first in the right ventricular outflow tract and thence in the right atrial cavity has been determined. Some preliminary results in two patients are shown in Tables I and II,

TABLE I  
*Cardiac Index During Atrial and Ventricular Pacing, and During Control Sinus Rhythm*

Rhythm	Cardiac Index (L./Min./M. <sup>2</sup> )	Ventricular Rate (/Min.)
NSR	3.01	74
V.P.	2.92	95
V.P.	2.35	105
V.P.	2.17	122
NSR	2.96	71
A.P.	3.12	96
A.P.	3.16	105
A.P.	3.10	122
A.P.	2.77	128

V.P. = Ventricular Pacing.

A.P. = Atrial Pacing.

*Diagnosis:* Obstructive pulmonary emphysema with cor pulmonale.

TABLE II  
*Cardiac Index During Atrial and Ventricular Pacing and During Sinus Rhythm*

Rhythm	Cardiac Index (L./Min./M. <sup>2</sup> )	Ventricular Rate (/Min.)
NSR	2.26	96
V.P.	1.73	99
V.P.	1.54	105
NSR	2.13	95
A.P.	2.20	108
A.P.	2.42	124
A.P.	2.87	125

*Diagnosis:* Obstructive pulmonary emphysema without cor pulmonale.

and these again demonstrate the important contribution of atrial systole toward maintenance of cardiac output. It is as yet too early to determine whether this pattern of output change with rate change is uniformly seen in man.

The role of atrial systole may also be emphasized by a consideration of the hemodynamic changes that accompany atrial fibrillation. Cardiac output decreases during atrial fibrillation (32, 39) and atrial flutter (40). At least some of this decrease in output may be caused by atrioventricular regurgitation

(21, 41, 42). Burchell has recently cautioned against overemphasizing the hemodynamic abnormalities caused by atrial fibrillation (33), and has quoted unpublished data of Williams and Wood demonstrating that there is no increase in regurgitant flow through the mitral valve during atrial fibrillation so long as a regular ventricular rate is maintained by electrical stimulation. There is little doubt however that the onset of atrial fibrillation often has serious hemodynamic and clinical consequences, especially in the presence of significant heart disease.

In summary therefore, some answers are available to the questions posed at the onset of this paper. There are definite hemodynamic abnormalities in patients with complete heart block. These abnormalities are at least partially corrected by increments in ventricular rate even during idioventricular pacing. Synchronization of atrial and ventricular activity is of further benefit for correction of the abnormal hemodynamic state in complete heart block. The role of atrial systole is probably more important in patients with heart disease than in subjects with normal hearts. It seems clear that the future of implanted pacemakers lies in the direction of synchronous atrio-ventricular pacemakers.

#### SUMMARY

The physiologic abnormalities produced by complete heart block have been analyzed. The effect of increases in heart rate upon these hemodynamic changes in complete heart block have been answered by a study of the data available from the literature as well as data from this laboratory. The potential additional benefits available from synchronization of atrial and ventricular activity have been reviewed. This analysis leads to the conclusion that synchronized atrio-ventricular pacemakers offer the best potential for correction of the hemodynamic abnormalities found in patients with complete heart block.

#### ADDENDUM

Since this paper was completed, the data of Segal and Hudson, previously mentioned only in preliminary form by Wade and Bishop (11), have been published in full (Segal, N., Hudson, W. A., Harris, P., and Bishop, J. M.: The Circulatory Effects of Electrically Induced Changes in Ventricular Rate at Rest and During Exercise in Complete Heart Block. *J. Clin. Invest.*, 43: 1541, 1964). Fourteen patients were studied, one with congenital and thirteen with acquired complete heart block. Measurements of cardiac output and right heart and systemic arterial pressures were made during the idioventricular rate, and at three higher rates, 50-65 beats/Min., 70-83 and 85-100 beats/Min., produced by right ventricular cavity pacing. Cardiac output generally rose (sometimes to normal levels) with increasing rate to reach a maximum at 70-83 beats/Min. However much as in the group of seventeen patients studied in our laboratory, various types of cardiac output responses were observed by Segal *et al.* In two patients cardiac output changed little as the ventricular rate was increased; in twelve a significant increase occurred. Rate increases



above 70–83 resulted in a fall in output in six patients; in three no change was observed and in five a slight increase (0.1–0.2 L./Min./M.<sup>2</sup> was noted). Stroke volume progressively fell with rate increments at all levels. Brachial and pulmonary arterial mean and diastolic pressures rose as the heart rate increased but systolic pressures changed little. Pulmonary artery wedge pressure was not altered as ventricular rate rose.

The same authors studied twelve patients during exercise at idioventricular rates and during catheter controlled heart rates of 50–100. The cardiac output was higher during the larger heart rates (cardiac index 3.0 and 4.0 at the idioventricularly and catheter stimulated rates respectively). However differences in oxygen consumption during the two exercise periods (354 and 415 ml./Min./M.<sup>2</sup> during the idioventricular and catheter exercise periods respectively) complicate the interpretation of the exercise output data. The arterial-mixed venous oxygen differences were 12.2 vol. per cent during exercise at the idioventricular rate (39 Min.) and only 10.8 vol. per cent during the higher rate (79 Min.) catheter controlled rate suggesting a true increase in cardiac output above and beyond that due to the larger oxygen consumption in the latter exercise period.

All in all these most recent data of Segal, Hudson *et al.* agree with most of the observations reported in the present review of this field.

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# Physiological Basis for Assisted Circulation\*

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The development of blood pumping techniques for "assisted circulation" is often regarded as a natural outgrowth of extracorporeal blood circulation and arterIALIZATION for the purpose of open-heart surgery, as it developed in the late 1950's. Indeed once the pump-oxygenator was recognized as an efficient clinical tool in cardiac surgery, several investigators proposed to use it for assistance to the failing heart. The rationale was that if pump oxygenators could replace the entire function of the heart and lungs for a few hours, a fortiori they should make a partial substitution of these organs possible. This approach to the problem of assisted circulation tends to make us forget that even before the advent of open-heart surgery, assisted circulation was envisioned from a quite different viewpoint. To mention but a few names, Brukhonenko (1) suggested a "parallel blood circulation" to complement pulmonary gas exchange. Dogliotti (2) employed perfusion techniques to prepare patients for surgery, thereby enabling them to withstand extensive thoracic operations. Salisbury (3) pioneered in extracorporeal blood circulation for the purpose of metabolic assistance during renal or hepatic failure.

The concept of assisted circulation nowadays implies three basic considerations: (a) Artificial organs and extracorporeal blood circuits are expected only to complement cardiac, respiratory or renal function. Thus the blood flow required is less than for open-heart surgery. Blood trauma is not a limiting factor, and circulatory assistance can be envisioned for periods of one or several days. (b) Since assisted circulation cannot yet be carried out indefinitely, the clinical indications are limited to reversible conditions, in which there is hope that temporary support may bridge over the acute phase of functional impairment of the heart, lungs, kidneys or liver. (c) Because assisted circulation is to be carried out while some degree of "normal" circulation is still present, the artificial organs must be employed in a way which does not burden or compete with the natural ones. On the contrary one must seek the proper harmonization of natural and artificial function (4).

These considerations make it clear that assisted circulation is a more complex undertaking than the simple, temporary replacement of the heart and lungs required for open-heart surgery. Incidentally, they may also explain why clinical attempts of assisted circulation have so far not met great success. While the usual scheme of circulation is still in action, assisted circulation interferes with a number of homeostatic mechanisms which normally maintain the integrity of the organism. With these procedures, much needs to be learned to

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solve or prevent technical difficulties which often outweigh the potential benefit of assisted circulation. All experimental data reported here were collected in normal animals, because of the necessity to investigate the results of assisted circulation procedures under physiologic conditions before applying them reasonably and with some chance of success to clinical situations.

Let us start by contrasting relatively simple emergency situations which constitute the accepted realm of resuscitation, with the less acute and more complex situations for which some form of assisted circulation might be considered (Table I). Indeed resuscitation already looks beyond the immediate problems of acute anoxia and cardiac arrest. In this light, assisted circulation may soon become a province of resuscitation. For that purpose however, it must involve more than extracorporeal pumping of blood. Several ancillary

TABLE I

*Patho-physiological Indications for a) Resuscitation; b) Assisted Circulation*

Resuscitation	Assisted Circulation
Apnea	Progressive asphyxia
Cardiac arrest	Chronic hypoxia
Exsanguinating hemorrhage	Chronic CO <sub>2</sub> retention
	Cardiac failure
	Cardiocirculatory shock
	Severe metabolic acidosis
	Renal coma
	Hepatic coma
	Exogenous intoxication
	Heat stroke
	Accidental hypothermia

techniques must be called upon to answer specific needs in terms of gas, water and solute exchange.

In the clinical conditions listed in Table I, assisted circulation can be analysed in terms of the primary need for assistance; failure of the heart to eject blood, failure of the circulatory system to distribute blood to the tissues, failure of the lungs to exchange gases adequately, failure of the organism as a whole to excrete waste products and to maintain its water and solute balance. Schematically, these are indications for what we call respectively cardiac assistance, circulatory assistance, respiratory assistance, and metabolic assistance. Obviously this scheme can be accepted only as a first approximation. The effect of the intended therapeutic maneuvers upon the organism as a whole needs always to be considered.

#### CIRCULATORY ASSISTANCE

In case of circulatory failure, the efficacy of mechanical assistance can be ascertained by a number of simple criteria: increase in arterial pressure, decrease in venous pressure, narrowing of the arterio-venous oxygen difference.

compensation of metabolic acidosis, improvement in the EEG, the ECG, or in urine flow. The most extensively studied technique for circulatory assistance is that of "veno-arterial pumping," which combines continuous phlebotomy with continuous arterial transfusion. With this procedure, part of the blood returning to the caval veins is shifted from the normal channels into an extracorporeal circuit, to be later infused into a peripheral artery. In normal subjects pulmonary blood flow is decreased in proportion to extracorporeal flow, yet systemic blood flow remains constant. The equipment required for veno-arterial pumping is remarkably simple and thus entails minimal blood trauma. The procedure can be carried out for several days (5). However, the return of venous blood to the femoral artery implies that the lower part of the body is perfused with blood of lower oxygen content, with the risk of hypoxic acidosis. In practice, it serves little purpose to carry more than 50 per cent of the basal cardiac output by veno-arterial pumping.

There is a situation where this metabolic limitation to veno-arterial pumping does not prevail. In some congenital heart malformations, fully saturated blood can be withdrawn from a cardiac cavity through a venous catheter, later infused directly into an artery to prevent mixing with poorly oxygenated blood. Specially designed cardiac catheters employed in conjunction with the McNeill-Collins pump carry blood flows in excess of 100 ml/min (Fig. 1). In puppies, perfusion can be carried out for 10 hours with virtually no blood damage. This technique may be employed in the cardiac catheterization laboratory for a short test of the potential value of assisted circulation.

If an artificial lung is inserted in an extracorporeal circuit for veno-arterial pumping ("veno-arterial oxygenation" or "partial heart-lung bypass"), pulmonary gas exchange is also supplemented (Fig. 9). In other words, partial heart-lung bypass substitutes for pulmonary blood flow while maintaining normal metabolic conditions in the organism (6). The new hemodynamic situation created by shunting blood from the central veins directly into the arterial tree is remarkably stable. Table II summarizes data collected in partial heart-lung bypass procedures of ten hours duration at a constant extracorporeal flow and constant blood volume. Pulmonary blood flow and right ventricular pressure decreased as soon as the extracorporeal circulation was activated and remained so until the bypass flow was arrested. The heart rate decreased to some extent. The aortic pulse pressure was more narrow than under control conditions. Under isovolumic conditions, the mean aortic pressure remained constant, indicating that there was no significant systemic vasomotor reaction.

The organism perfused from vein-to-artery is exceedingly sensitive to small changes in blood volumes. At constant extracorporeal flow rate, a small blood loss causes a marked decrease in cardiac output. Similarly, a moderate increase in blood volume enhances cardiac output beyond what is observed in the intact circulation (Fig. 2). Apparently a heart made to pump abnormally low outputs, decreased in size as a consequence of a vein-to-artery shunt, yet receiving its normal coronary blood supply, is unusually sensitive to changes in diastolic filling pressure. This response is characteristic of a circulation ade-



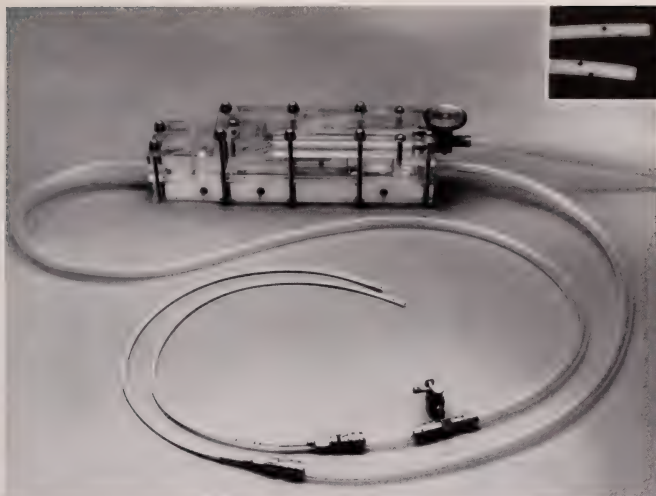


FIG. 1. Pump (McNeill-Collins) and catheters (Electro-Catheter Corp., Metuchen, New Jersey) employed in a no-prime circuit for veno-arterial pumping in the cardiac catheterization laboratory.

TABLE II

	Before Bypass	Just After Start	Midway	Just Before End	After Bypass
$Q_E$ .....	0	$35 \pm 5$	$35 \pm 5$	$35 \pm 5$	0
$Q_L$ .....	$92 \pm 30$	$51 \pm 21$	$51 \pm 20$	$55 \pm 30$	$107 \pm 31$
$Q_T$ .....	$92 \pm 30$	$86 \pm 22$	$86 \pm 21$	$90 \pm 32$	$107 \pm 31$
Heart rate.....	$161 \pm 38$	$144 \pm 39$	$143 \pm 35$	$159 \pm 38$	$155 \pm 40$
SVP.....	$6 \pm 4$	$5 \pm 3$	$5 \pm 3$	$4 \pm 3$	$5 \pm 4$
RVP.....	$23 \pm 7$	$18 \pm 7$	$17 \pm 5$	$19 \pm 8$	$24 \pm 6$
SAP.....	$110 \pm 22$	$114 \pm 24$	$120 \pm 21$	$113 \pm 26$	$122 \pm 24$
Pulse pressure.....	$29 \pm 9$	$23 \pm 9$	$22 \pm 7$	$22 \pm 10$	$35 \pm 8$

Mean value ( $\pm$  one standard deviation) of several hemodynamic parameters in veno-arterial oxygenation procedures. Data were collected in 26 dogs just prior to perfusion, in the first 30 minutes of the procedure, then again five hours later, in the last 30 minutes of perfusion, finally about one hour after termination of bypass.  $Q_E$  = extracorporeal blood flow, in ml/kg/min;  $Q_L$  = pulmonary blood flow, in ml/kg/min;  $Q_T$  = total, or systemic blood flow, in ml/kg/min; SVP = central venous pressure, in cm H<sub>2</sub>O; RVP = systolic right ventricular pressure, in mm Hg; SAP = mean systemic arterial pressure, in mm Hg. (Unpublished data of M.A. Hopf and P. M. Galletti.)

quately supported regardless of the actual contribution of the cardiac output and of the extracorporeal circuit. In case of failure or decay of the preparation, myocardial damage and dilatation of the cardiac cavities tend to obscure this remarkable mechanism.

In the practice of partial heart-lung bypass, because the caval veins are usually connected by a fixed resistance catheter to an extracorporeal reservoir open to atmospheric pressure, any change in central venous pressure tends to translocate blood from the inner circuit into the extracorporeal circuit or conversely. Stability is best achieved when the pump-oxygenator not only provides for a constant venous pressure, but is also "Bainbridge-minded" or "Starling-

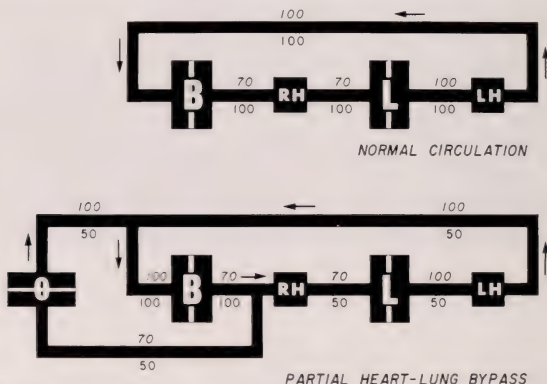


FIG. 2. Block diagram of normal circulation and partial heart-lung bypass circulation in the dog. B: systemic capillary beds; RH: right heart; L: pulmonary capillary bed; LH: left heart; O: pump-oxygenator. Italic figures: standard value for blood oxygen saturation in percent. Upright figures: standard value for blood flow rate, in ml/kg/min.

minded," meaning that it reacts to any increase in extracorporeal venous drainage by a corresponding increase in rate or stroke volume of arterial infusion (7). A transfusion bag employed as a closed venous reservoir goes a long way to provide the required stability, because it opposes variations in central venous pressure, stores blood in the case of veno-constriction, and in case of sudden blood loss by the organism, furnishes to the heart the amount of venous return needed to prevent a decrease in cardiac output (Fig. 3).

We recently attempted to utilize our best knowledge of perfusion techniques in carrying out partial heart-lung bypass for 24 hours in normo-thermic dogs breathing spontaneously. The extracorporeal circuit consisted of a two square meter Teflon membrane oxygenator (Klung) and two roller pumps, primed with a saline-dextrose-dextran solution. The extracorporeal flow was uniformly set at 600 ml/min, so as to take over about 30 per cent of the cardiac output and pulmonary gas exchange. Eight out of eleven animals were chronic sur-

vivors. Blood damage remained well within acceptable limits, with an average final plasma hemoglobin of 160 mg%, and a final platelet count of 120,000  $\text{mm}^3$ . No abnormal bleeding or clotting tendency was observed in the post perfusion period. Tests of hepatic and renal function remained normal. The maintenance of an adequate metabolic balance (pH,  $\text{pCO}_2$ , Cl, Na, K and glu-

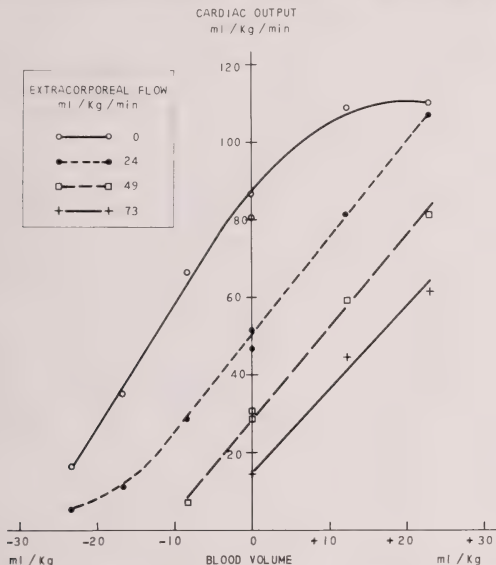


FIG. 3. Relationship between pulmonary blood flow (= cardiac output) and body blood volume at various levels of partial heart-lung bypass. The thermodilution technique was employed for serial measurements of cardiac output in a dog, first under control conditions, then at increasing rates of extracorporeal flow. The blood volume was altered between measurements by allowing a controlled amount of transfusion from, or exsanguination into, the extracorporeal circuit. A steady blood volume was maintained for at least 5 minutes prior to each determination of cardiac output. Each experimental point is the average of 5 thermodilution curves. (Unpublished data of E. Lüthy and P. M. Galletti.)

cose levels) meant that the administration of any drug was seldom required. These observations indicate that adequate equipment for long-term perfusion is presently available. Extracorporeal blood circulation and oxygenation can be maintained safely for clinically useful periods with simple equipment requiring no priming blood.

#### CARDIAC ASSISTANCE

The concept of mechanical assistance to the heart itself is based on the postulate that the failing myocardium may benefit from a period of relative

"rest," i.e., of decreased energy expenditure. The rationale for this is that failure of the heart to pump blood in sufficient amounts results from an imbalance between energy supply to the myocardium and energy expenditure required from the heart in order to maintain the circulation. It is assumed that any external maneuver which decreases the "work load" of the myocardium while providing satisfactory blood distribution to the tissues helps to relieve cardiac failure. This attractive concept finds some support in the classical observation of improvement of cardiac failure following a period of strict bed rest. Yet it is important to stress that so far there is no direct experimental evidence of reversal of cardiac failure by mechanical assistance persisting beyond the phase of assistance. The present state of affairs is undoubtedly

TABLE III

*List of the Physical Determinants of Cardiac Work and Cardiac Efficiency*

Factors Invoked in the Estimation of the "Work Load" of the Heart	
Kinetic energy factor	Ventricular stroke volume $\times$ Mean velocity of flow <sup>2</sup> $\times$ Heart rate
Potential energy factor	Ventricular stroke volume $\times$ Mean arterial pressure $\times$ Heart rate
Wall tension factor	Ventricular end-diastolic radius $\times$ Ventricular mean systolic pressure
Overall indices	Time Tension Index Ventricular function curves End-diastolic ventricular pressure Rate of isovolumetric contraction Myocardial contractile force Myocardial oxygen uptake Ratio of ventricular end-diastolic volume to mean systolic ventricular compliance

associated with the difficulty of producing in the experimental animal a type of cardiac failure which can be compared to that in man. On the other hand, clinical attempts of mechanical assistance to the failing heart have usually involved complex situations where a correction of the circulatory disturbances was likely to overshadow any true improvement of the pumping mechanism itself.

Another difficulty in attempting to solve the problem of cardiac assistance experimentally stems from the lack of agreement among cardiac physiologists as to what constitutes the physical determinants of cardiac work (Table III). Depending upon which criterion is selected, almost any procedure of assisted circulation may be proven useful or detrimental in terms of the work load of the heart. Considering the shortage of significant information, it is quite pointless to debate the theory of cardiac assistance much further.

It may be more informative at this time to study the effects of extracorporeal circulation procedures on various aspects of cardiac mechanics. As an example Figure 4 schematizes the change in Time Tension Index (TTI) observed when a moderately hypotensive circulation is supported by (a) veno-arterial pumping; (b) diastolic augmentation; (c) synchronized counterpulsation. Veno-arterial pumping using a continuous flow pump can only re-establish a normal arterial pressure at the cost of an increased TTI. Diastolic augmentation, a group of procedures attempting to boost diastolic run off by a pumping action limited to mid-diastole, enhances blood distribution to the

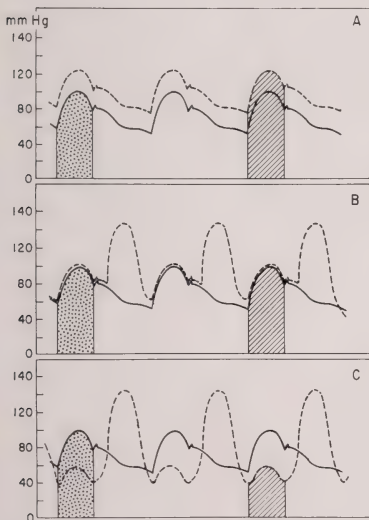


FIG. 4. A schematic diagram of the effects of veno-arterial pumping (A), diastolic augmentation (B), and synchronized counterpulsation (C), upon the aortic pressure, left ventricular pressure and left ventricular Time Tension Index. The solid line represents the contour of the aortic pressure curve in a case of moderate hypotension. The dotted line depicts the aortic pressure curve when mechanical assistance to the heart is carried out. The dotted area represents the Time Tension Index when the blood pump is off. The hatched area shows the Time Tension Index when the blood pump is on. [Redrawn from Birtwell *et al.* (8).]

tissues without affecting the systolic TTI. Synchronized counterpulsation, as provided by an ectopic "ventricle" which sucks blood from the aorta during systole and returns it during diastole, achieves a rise in mean arterial pressure with a decrease in TTI. Thus from the theoretical standpoint of Time Tension Index, synchronized counterpulsation is the technique of choice for assistance to the failing heart (8). Unfortunately, it meets practical difficulties in the synchronization of the external ventricle with the electrocardiogram. Moreover, peripheral cannulation often offers too much resistance to permit an adequate stroke volume during the short interval of the cardiac cycle available for pumping (9). For all these reasons, it has been impossible so far to apply synchronized counterpulsation for prolonged periods of time.

There is limited information available about the effect of cardiac assistance procedures upon left end-diastolic ventricular pressure and left atrial pressure, because of the difficulties associated with the reliable determination of these pressures in the closed-chest preparation. However, there is little change to be expected in the normal animal, and much debate as to the significance of elevated diastolic ventricular pressure as an index of cardiac "failure." It has been demonstrated that left atrial pressure is always significantly decreased whenever partial heart-lung bypass carries more than 25% of the total blood flow (10). The same is probably true with left heart bypass. The decrease in left atrial pressure does not necessarily influence ventricular dynamics, since the ventricle generates essentially the same pressure whether it ejects a large stroke volume, or reduces its output to intermittent "emptying beats," or performs an "isovolumetric exercise" without even opening the aortic valves (9).

The situation is somewhat confused as to the effect of cardiac assistance on myocardial oxygen uptake. With partial heart-lung bypass, Salisbury (10) observed that in some experiments the oxygen consumption of the heart decreased only when the cardiac output was reduced to zero; in other experiments, a marked reduction of cardiac oxygen use was evident when the extracorporeal circuit carried over 50% of the systemic flow. With left heart bypass, Schenk *et al.* (9) also reported an inconsistent reduction in myocardial oxygen uptake. Left heart bypass had to be complete to cause a decrease in left ventricular diameter and in coronary flow.

Not considered in the above discussion are the effects of cardiac assistance on heart rate and heart size, which are also important factors of myocardial energy expenditure. Partial heart-lung bypass causes a decrease in heart rate if care is taken to maintain the body blood volume constant (Table I). The reduction in heart rate is even more apparent if blood volume is decreased as the extracorporeal flow rate is increased (11), particularly when cardiac action was initially rapid because of circulatory overload. Isovolemic partial heart-lung bypass also causes a lasting decrease in end-diastolic ventricular volume (Fig. 5). The decrease in end-diastolic volume is more marked for the right ventricle, which then pumps against a reduced pulmonary artery pressure, than for the left ventricle, which still has to overcome an essentially normal aortic pressure (12). Here again the reduction in the size of the ventricular cavities is only observed in hearts which have not been damaged by extensive "plumbing" and when hypervolemia is carefully avoided as the pump is started. These examples illustrate that mechanical assistance can effect the heart in several ways, potentially beneficial or detrimental. Details of technique not always appreciated by the investigator, much less mentioned in scientific reports, often negate the physiological aim of the assistance procedure.

#### RESPIRATORY ASSISTANCE

The efficacy of respiratory assistance through extracorporeal blood arterialization is readily demonstrated, since a narrowing of the arteriovenous oxygen difference, the correction of respiratory acidosis and in the case of chronic



tissue hypoxia the compensation of metabolic acidosis are obvious criteria. With the technique of partial heart-lung bypass the total amount of arterialized blood distributed to the body tissues is the sum of pulmonary blood flow plus extracorporeal flow. As the venous return to the caval veins is progressively shifted from the normal channels into the extracorporeal circuit, the artificial lung takes over the function of the natural lung. Provided enough

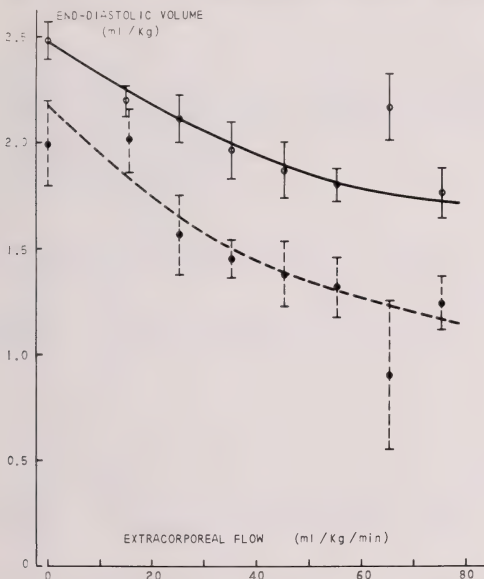


FIG. 5. Ventricular end-diastolic volume, in ml/kg, as a function of extracorporeal flow rate, in ml kg min. The solid line corresponds to the left ventricle, the broken line to the right ventricle. Ventricular end-diastolic volume was measured by the thermodilution technique of Lüthy. Circle and dots represent the mean values. The vertical bars correspond to plus or minus one standard error. [Redrawn from Salgado *et al.*, (12).]

blood can be drained into the extracorporeal circuit, the artificial lung will compensate for any degree of functional impairment of the natural lung. The adequacy of oxygen distribution is confirmed by the observation of a steady venous oxygen saturation and arterial blood pH over periods as long as 10 or 24 hours.

Partial suppression of the gas exchange function of the natural lung appears to cause a decrease in respiratory minute volume. In other words, pulmonary ventilation decreases as pulmonary blood flow is reduced by an increase in

extracorporeal flow (6). This respiratory depression disappears where pulmonary blood flow is reduced to very low values, and is then replaced by a marked hyperventilation. Figure 6 illustrates how rapidly pulmonary ventilation decreased at the beginning of perfusion when the pulmonary blood flow was suddenly reduced to 20% of its control value. A few minutes later, the pulmonary blood flow was further reduced to about 10% of control, by permitting the animal to loose 100 ml of blood into the extracorporeal circuit. This led to a sustained hyperventilation, which subsided when pulmonary blood volume and

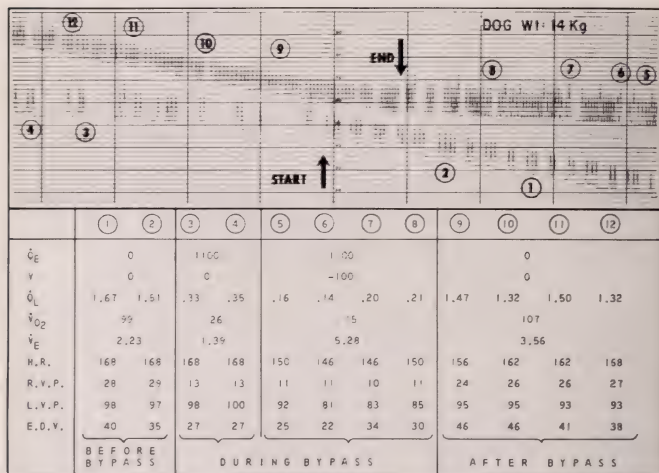


FIG. 6. Effects of partial heart-lung bypass at high extracorporeal flow rate ( $\dot{Q}_E$ , in ml/min) at normal or decreased blood volume ( $\dot{V}$ , in ml/min) upon pulmonary blood flow ( $\dot{Q}_L$ , in L/min), pulmonary oxygen uptake ( $\dot{V}_{O_2}$ , in ml/min), pulmonary ventilation ( $\dot{V}_E$ , in L/min), heart rate (H.R.), systolic right ventricular pressure (R.V.P., in mm Hg), systolic left ventricular pressure (L.V.P., in mm Hg) and left ventricular end-diastolic volume (E.D.V., in ml). Vertical lines on the spirometer recording-time in minutes.

flow were brought back to normal by arresting the external perfusion and returning the 100 ml of blood to the vascular system.

The technique of partial heart-lung bypass, whereby pulmonary circulation and extracorporeal circulation are set in parallel (Fig. 2) may appear the most physiological one for the purpose of complementing pulmonary gas exchange. However, circuits with the artificial lung located in series with the natural lung have also proved adequate for respiratory assistance. Furthermore, there is no need to blindly imitate nature, which performs most exchange functions in a capillary bed located between artery and vein. Under specific conditions, artificial lungs are best employed in an artery-to-artery, vein-to-

artery or vein-to-vein shunt. In the case of veno-venous oxygenation (Fig. 7) blood is drained from a caval vein, arterialized outside the body, and returned to the right atrium, to then follow the usual circulatory channels. The procedure has no significant hemodynamic effect. In terms of gas exchange, it is difficult to collect all the venous blood into the extracorporeal circuit and to prevent it from entering the pulmonary circulation before undergoing extracorporeal arterIALIZATION. Because the two streams cannot be completely sepa-

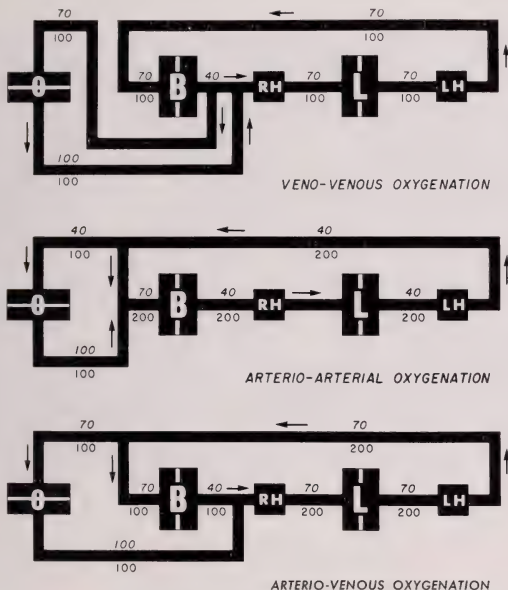


FIG. 7. Block diagram of veno-venous oxygenation, arterio-arterial oxygenation and arterio-venous oxygenation in the absence of pulmonary gas exchange. Symbols as in Fig. 2.

rated, veno-venous oxygenation can only partially replace pulmonary gas exchange in an animal breathing room air. This is no longer true when the animal is made hypoxic by breathing a low oxygen mixture. An example of complete substitution for pulmonary gas exchange by means of veno-venous oxygenation is depicted in Figure 8. Following control measurements, the extracorporeal blood flow was increased stepwise, to a value corresponding to the basal cardiac output. This decreased the pulmonary oxygen uptake by about 40%. At that point, the oxygen-filled spirometer from which the animal was breathing was replaced by another one, with no soda lime absorber

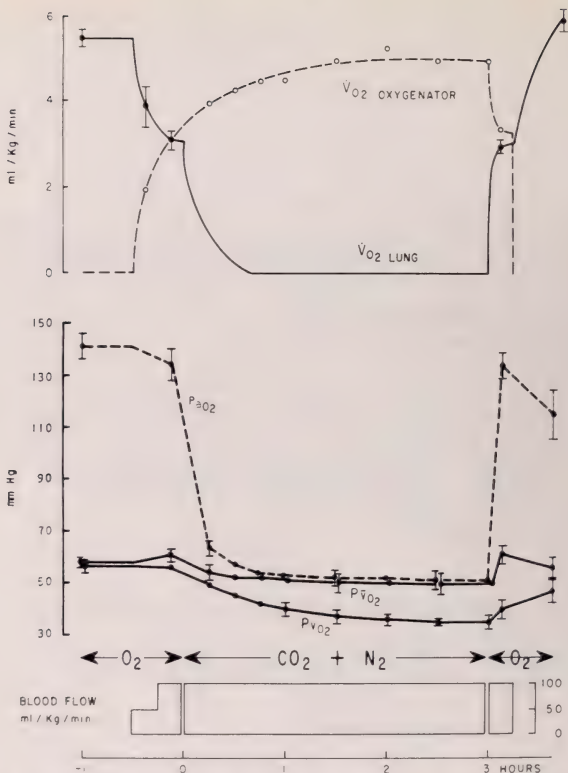


FIG. 8. Pulmonary oxygen uptake ( $\dot{V}_{O_2, \text{LUNG}}$ , in ml kg min), extracorporeal oxygen uptake ( $\dot{V}_{O_2, \text{OXYGENATOR}}$ , in ml kg min), arterial blood oxygen tension ( $P_{aO_2}$ , in mm Hg), mixed venous oxygen tension ( $P_{\bar{v}O_2}$ , in mm Hg) and peripheral venous oxygen tension ( $P_{vO_2}$ , in mm Hg) before, during and after veno venous oxygenation. Once the blood flow in the vein to vein shunt (block diagram at bottom) was established at a level close to the control cardiac output, pulmonary gas exchange was made impossible by connecting the tracheal tube to a rebreathing circuit. A steady state of pre-pulmonary blood arterialization was achieved. (Unpublished data of F. J. Martinez and P. M. Galletti.)

and filled with 7%  $CO_2$  in nitrogen. The alveolar oxygen tension decreased progressively until pulmonary gas exchange became negligible, as indicated by equal gas tensions in pulmonary arterial blood, alveolar gas and systemic arterial blood (Fig. 8). Meanwhile, the extracorporeal lung, in the veno-venous

circuit, took over the gas exchange function. Oxygen uptake and distribution to the tissues were simply shifted to a lower portion of the oxygen dissociation curve, with pH and  $p\text{CO}_2$  kept within normal limits by adequate ventilation of the artificial lung. The amount of oxygen provided at the prepulmonary level was calculated as the product of extracorporeal flow times the difference in oxygen content between blood returned to the body and blood drained from it. It stabilized at values corresponding to the oxygen uptake measured under control conditions. Quite similar data were obtained with arterio-venous oxy-



FIG. 9. Veno-arterial oxygenation in the newborn lamb. Venous-blood was collected from the right atrium through a cannula inserted in the external jugular vein. A carotid artery and a femoral artery were cannulated. Alternately one artery served for arterial infusion and the other for arterial pressure and monitoring. (Unpublished experiments of P. M. Galletti, E. C. Peirce and M. Orzalesi.)

genation in hypoxic animals. These observations point to a fundamental principle of respiratory assistance, namely that the organism requires a certain volume of oxygen per unit of time, but cares much less for the site where this oxygen is introduced into the blood (i.e. in, before, or after the natural lungs), or for the tension at which the oxygen is distributed to the tissues.

Another type of deviation from the natural conditions of oxygen supply was investigated in premature lambs delivered by cesarean section. The lambs were initially kept connected to their placentae, and prevented from breathing. Cannulae were inserted for veno-arterial oxygenation in a one square meter Klung membrane oxygenator at a flow rate of about 120 ml/kg/min (Fig. 9). The umbilical cord was then tied, and the lamb suddenly passed from the

fetal conditions of oxygen supply to the tissues at relatively low tension to a distribution and intake higher up on the oxygen dissociation curve. With the artificial lung in a vein-to-artery shunt, rather than in an artery-to-vein shunt as the natural placenta, the site of extracorporeal gas exchange was located in series with the systemic capillary beds, a more adequate approach to complete pulmonary substitution than the mere replacement of the placenta in the site and circuit of the natural organ (Fig. 10). Stable metabolic conditions were observed for several hours, and the animals made no attempt to breathe. Following arrest of extracorporeal oxygenation, and a short period of hypoxia, hypotension and bradycardia, the lambs started to ventilate rapidly, gained consciousness and survived their delayed birth without apparent ill effect.

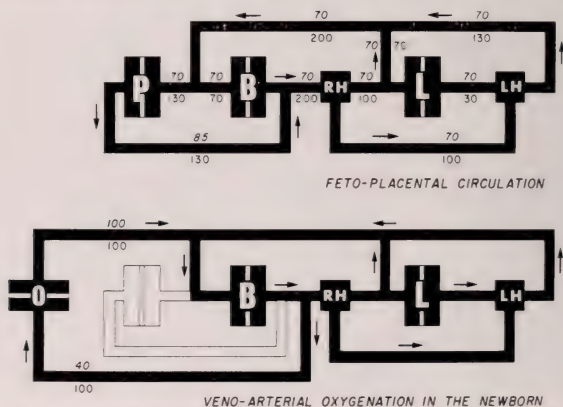


FIG. 10. Block diagram of feto-placental circulation, and of veno-arterial oxygenation in the asphyxiated newborn. Symbols as in Fig. 2.

This demonstrates that techniques for complete respiratory support of the anoxic newborn are now available. Whether these techniques will prove useful in clinical conditions remains to be investigated.

#### METABOLIC ASSISTANCE

The clinical conditions which may benefit from assisted circulation involve more metabolic disorders than plain hypoxia. Beside acidosis, one observes disturbances of water and electrolyte balance, and also accumulation or depletion of some important plasma solutes. These deviations can sometimes be controlled by the administration of drugs. However, their complexity is such that hemodialysis often represents the most satisfactory approach.

In the field of assisted circulation, the artificial kidney serves for ultrafiltration of edema fluid. Hemodialysis can also provide a rapid correction of acid-



base and electrolyte imbalance, without the need for multiple biochemical determinations. The design of a heart-lung kidney or Klung (Fig. 11) was proposed to combine oxygenation with dialysis. The Klung not only arterializes blood; it also permits continuous clearance of undesirable substances from the plasma while maintaining others within the optimal range. Judiciously em-

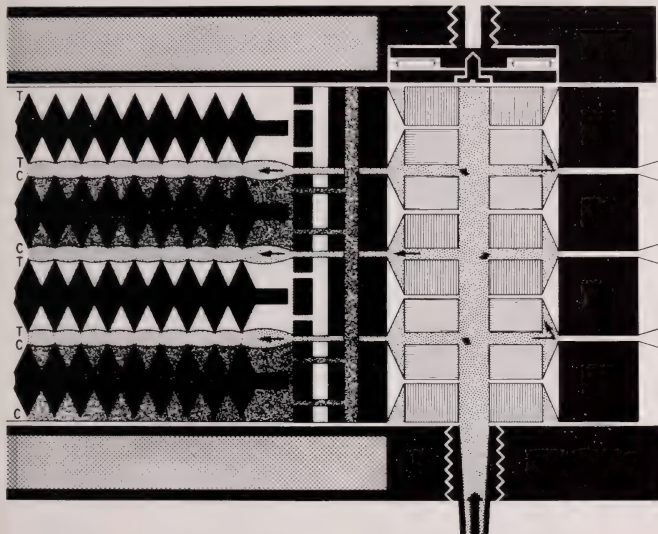


FIG. 11. Scheme of the "Klung" membrane lung kidney. Blood (large dots) is distributed in a number of parallel films through a column of discs (hatched) with a hole in center and radial holes. Each blood film spreads between a teflon membrane (T) for gas exchange and a cellophane membrane (C) for water and solute exchange. The membranes are supported by opposed cone fields molded in a sheet of silicone rubber (black). Oxygen (small dots) proceeds from a distribution manifold into the valley between the cones, so as to sweep the outside of the teflon membranes. The dialyzing solution (gray) comes in contact with the cellophane membranes only. The assembly of membranes, supporting mats and distributing discs is stacked and compressed in an aluminum frame, which also serves for thermostabilization (cross hatched).

ployed, the combined heart-lung-kidney is a more effective perfusion tool than the simple heart-lung machine (13). Since it adds little to the complexity of the extracorporeal circuit, it will probably replace, for the purpose of assisted circulation, the conventional pump-oxygenator.

With conventional hemodialysis, the correction of plasma solute level is passive, that is restricted to those solutes for which a concentration gradient can be created with respect to the dialysate. Several techniques have been proposed to gain active control of plasma solutes levels. The expression "arti-

ficial liver" or "artificial placenta" have been coined for that purpose. The "artificial liver" usually denotes hemodialysis equipment in which animal's liver ground, sliced or intact and perfused, has been added to the dialysate. There is little evidence as yet for the usefulness of this technique. The procedure in which the dialysate is replaced by the blood of a live animal circulating in closed circuit from and to a donor organism, should properly be called "cross-dialysis" or "parabiologic dialysis" (14). Cross dialysis truly represents active hemodialysis, since it takes advantage of the clearing capacity of the

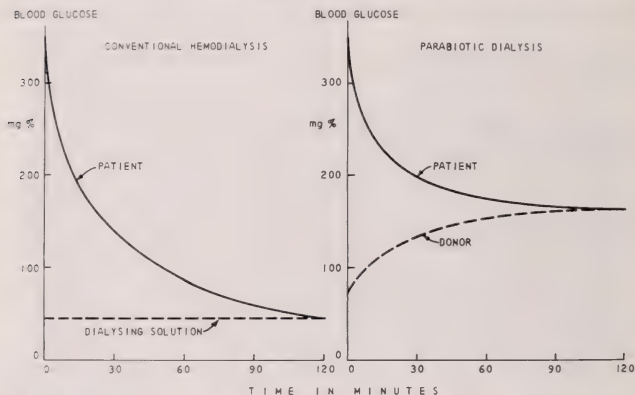


FIG. 12. Blood glucose levels in a chronic pancreatectomized dog treated a) by conventional hemodialysis; b) by parabiologic dialysis using a healthy sheep as the "donor". In both experiments, a 2 square meter Cuprophane Kling kidney was employed in an artery-to-vein shunt. Recipients blood flow: 200 ml/min; dialysate (or donor blood) flow: 800 ml/min. Since the dialysate was not recirculated the patient's glucose levels decreased steadily until it reached the level established in the rinsing solution (45 mg%). During parabiologic dialysis the recipient's glucose levels did not fall as sharply as with conventional dialysis, because the donor's blood glucose level started at a relatively high value, and increased as glucose was transferred from the recipient into the donor. (Unpublished data of R. W. Alexander and P. M. Galletti.)

donor organism (liver, kidneys, and reticulo-endothelial system) to correct blood disturbances in the patient. Modern membranes permit significant exchange of biochemical compounds and drugs with molecular weight up to at least 6,000. Thus cross-dialysis offers many of the advantages of cross-circulation, without the difficulties involved with the latter technique, namely blood volume control and erythrocyte compatibility problems. Because the concentration gradients between patient's and donor's blood are relatively small, cross-dialysis is less efficient than conventional hemodialysis in terms of rapid clearing of unwanted substances. However this is offset by two major advantages: first correction and equilibration extend to all plasma components, not only to those dissolved in the dialysate as is the case with the artificial

kidney, second overcorrection cannot occur, since plasma levels in the healthy donor organism set a natural limit to solute exchange. Figure 12 illustrates the time course of correction of blood glucose levels in a pancreatectomized dog treated once by conventional hemodialysis and another time by cross-dialysis with a healthy sheep. In both cases, the hyperglycemia was corrected within one hour, without injection of any drug.

The combination of artificial blood oxygenation with parabiotic dialysis (Fig. 13) represents the closest approximation to an artificial placenta available today. In spite of the relative complexity of the circuitry, this technique

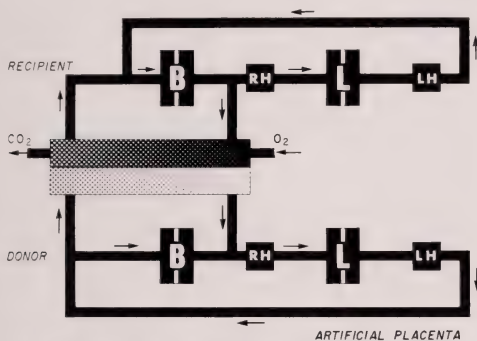


FIG. 13. Block diagram of "artificial placenta", or combination of veno-arterial oxygenation (upper part of diagram) plus parabiotic dialysis with a recipient organism (lower part of diagram). The exchange unit in the center is a combined lung-kidney which simultaneously exchanges oxygen, carbon dioxide, water and solutes.

will hopefully permit extension of perfusion procedures beyond the present limit of one or two days.

#### CONCLUSION

The main conclusion to be drawn from laboratory experience is that all perfusion procedures have advantages and drawbacks (15). If one disregards the question of technical complexity and concentrates on physiological problems, he will conclude that most available techniques have potential applications in one disease or another. However, all techniques have also some limitations and some absolute contraindications. Table IV summarizes our present views as to the beneficial ( $++$  or  $+$ ) or potentially detrimental ( $-$  or  $--$ ) effects of various perfusion procedures in the four main classes of "failure" outlined in the introduction. At this point in the development of techniques for "mechanical assistance to the failing heart," there is still a need for extensive laboratory investigations. Pilot clinical studies are justified for those who have the experience, the equipment and the hospital facilities required for such in-

TABLE IV

*List of Mechanical Procedures Proposed for Assisted Circulation with Special Reference to Their Beneficial (+) or Potentially Detrimental (-) Effect on the Various Aspects of Circulatory Failure*

Procedure	Cardiac Effect	Circulatory Effect	Respiratory Effect	Metabolic Effect
Right heart bypass	++	++	0	0
Left heart bypass	++	++	0	0
Veno-arterial pumping	+ to -	+	-	--
Veno-arterial pumping with aortic compartmentalization	+	+	-	-
Partial heart-lung bypass	++ to -	++	++	+
Diastolic augmentation	+	+	0	0
Diastolic balloon pumping	+	+	0	0
Aortic bypass pumping	+	+	0	0
Aortic sleeve pumping	+ to -	+	0	0
Ectopic ventricle counterpulsation	++	+	0	0
External counterpulsation	+ to -	+ to -	0	0
Synchronized airway pulsation	+ to -	+	+	0
Veno-venous oxygenation	0	0	++	+
Arterio-venous oxygenation	-	-	++	+
Arterio-arterial oxygenation	0	0 to +	++	+
Extracorporeal dialysis	0 to -	0 to -	0	++
Parabiotic dialysis	0 to -	0 to -	0	++
Extracorporeal ultrafiltration	+	0	0	+
Artificial heart-lung-kidney	++ to -	++	++	++

vestigations. Hopefully, the use of mechanical hearts, lungs and kidneys will open a new era in the treatment of conditions which resist conventional drug therapy and claim numerous lives.

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## Foreword

These papers, contributed by the Departments of Hematology, Physics, and Medicine, bring together dynamic studies dealing with certain phases of iron and iodine metabolism.

The nature of the problem requires methodological approaches of a high level of sophistication. It is realized that not all readers will be able to follow in detail the complex mathematics necessary to analyze the multiple simultaneous processes involved. In spite of this, it is felt that such a presentation will serve a useful purpose in bringing to the attention of a general medical audience an area of clinical investigation that is becoming more important each day. Perhaps, even more significant than any quantitative results obtained, is an appreciation of the general approach to the problems of multiple pool analysis. It is hoped that these papers will fulfill that need.

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# Multiple-Pool Analysis in Tracer Studies of Metabolic Kinetics: I

## General Considerations and Solutions of Simpler Systems (One and Two Pools)\*

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### DEFINITIONS AND GENERAL DISCUSSION OF MULTIPLE-POOL SYSTEMS

In studying the normal physiological behavior of any given substance, we are concerned with both its static and dynamic aspects, i.e., with its spatial distribution and chemical states, as well as with the rates of transfer from one such localization or state to another. When a metabolic system is in a steady state, i.e., dynamic equilibrium, the basic substance enters and leaves any particular chemical or spatial phase at essentially constant rates. The quantitative distribution of the substance remains nearly constant, except for such regular or cyclic variations as may be due to normal changes in the general physiological state of the organism. In general, quantitative chemical studies give static results and are limited either to measurements of intake and excretion or to direct observations regarding concentrations in accessible body compartments. Therefore, direct chemical analysis can yield little information about the dynamic structure of such systems. Theoretically the dynamics of a substance can be studied by increasing or decreasing the metabolic content of a given compartment, or by interfering with the metabolic pathway of the substance. However, any attempts which involve such alterations disturb the initial steady state and, therefore, introduce extraneous factors.

Hence the need for more physiological methods of investigation, such as tracer techniques. Tracers fulfill three important requirements. Firstly, they are as a rule biologically indistinguishable from the substance under investigation, and, therefore, may be assumed to undergo the same metabolic changes. Secondly, they can be administered in such small amounts as not to disturb the dynamic equilibrium of the system as a whole. And finally since tracers are not in a steady state when introduced instantaneously into one part of the system, they exhibit quantitative changes which can be mathematically analyzed and described as a function of time. These changes reflect the various rates of reactions and transfers of the physiological (non-tracer) metabolite under investigation.

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In practice, experimental tracer data are subject to considerable random as well as systematic errors, and their graphic representation can be approximated by an infinite variety of mathematical curves. Therefore, before analyzing such quantitative tracer data, certain assumptions and definitions about the system under consideration must be made. Unless stated otherwise, the following definitions and assumptions apply both to tracer and non-tracer material (see Fig. 1).

$Q_i$ : Each system consists of one or more pools,  $Q_i$ , defined as distinct aggregates of the basic substances; these aggregates may differ from each other by virtue of their anatomic location, or differences in chemical properties of the form in which the substance occurs.  $Q_i$ , in addition to denoting the  $i^{\text{th}}$  pool, represents the total amount of the substance in the pool  $Q_i$ .

$k_i$ : The substance considered leaves each of the pools  $Q_i$  with a constant positive relative rate  $k_i$ , i.e., the amount  $k_i Q_i$  leaves pool  $Q_i$  per unit time. This

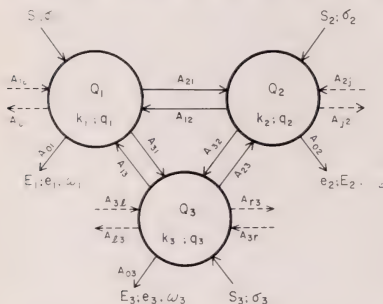


FIG. 1. Schematic representation of three of the pools of an  $n$ -pool system ( $n > 3$ ).

defines the relative rate  $k_i$  as a multiple or fraction of the total quantity of the metabolite in the pool  $Q_i$  which leaves the given pool per unit time.

$A_{ji}$ : A constant fraction,  $A_{ji}$ , of the amount  $k_i Q_i$  leaving the pool  $Q_i$  is transferred to another pool  $Q_j$  at the absolute rate  $A_{ji} k_i Q_i$ .

$A_{oi}$  and  $E_i$ : A constant fraction,  $A_{oi}$ , of the amount  $k_i Q_i$  that leaves pool  $Q_i$  is eliminated entirely from the  $n$ -pool system at an absolute rate  $A_{oi} k_i Q_i = E_i$ . The sum of all fractions of the metabolite ( $A_{oi}$  and  $A_{ji}$ 's) leaving pool  $Q_i$  must be equal to 1: in an  $n$ -pool system the sum

$$\sum_{\substack{j=0 \\ j \neq i}}^n A_{ji} = 1.$$

In a three-pool system this reduces to

$$A_{o1} + A_{21} + A_{31} = A_{o2} + A_{12} + A_{32} = A_{o3} + A_{13} + A_{23} = 1.$$

$\omega_i$ :  $\omega_i$  denotes the fraction of the total amount of non-tracer material leaving

the system which leaves through pool  $Q_i$ :

$$\omega_i = \frac{E_i}{\sum_1^n E_j} \quad \text{and} \quad \sum_1^n \omega_i = 1.$$

In a three-pool system

$$\omega_1 = \frac{E_1}{E_1 + E_2 + E_3}, \text{ etc.}$$

$S_i$ :  $S_i$  is the constant absolute rate of supply of the non-tracer substance to the system through pool  $Q_i$  ( $S_i$  may be considered as coming from a source exogenous to the system).

$\sigma_i$ :  $\sigma_i$  denotes the fraction of the total amount of non-tracer material entering the system which enters through pool  $Q_i$ :

$$\sigma_i = \frac{S_i}{\sum_1^n S_j} \quad \text{and} \quad \sum_1^n \sigma_i = 1.$$

$q_i = q_i(t)$ :  $q_i = q_i(t)$  stands for the amount of tracer contained in pool  $Q_i$  at the time  $t$ . (In case the tracer is radioactive, all  $q_i(t)$ 's represent the amounts of isotope corrected for physical decay with reference to some standard time.)

$q_o = q_i(o)$ :  $q_o = q_i(o)$  is the amount of tracer which is introduced instantaneously into the accessible pool  $Q_i$  at the initial time  $t = o$ .

$e_i$ : The fraction of  $q_o$  which is eventually eliminated from the system with  $E_i$  is designated by  $e_i$ :

$$e_i = \frac{A_{oi}k_i}{q_o} \int_0^\infty q_i(t) dt.$$

All mixing is assumed to be instantaneous and therefore  $q_i$  is subject to the same relative rates as  $Q_i$ . In a strict sense, the assumption of instantaneous mixing is not always necessary and may be replaced by the more general condition that the  $q_i$ 's be subject to the same relative rates of change as the  $Q_i$ 's. (See discussion in "Multiple-Pool Analysis in Tracer Studies of Metabolic Kinetics: II.")

Implicit in the above definitions is the basic assumption of first order reactions, i.e., the absolute rates of transfer from the  $i^{\text{th}}$  pool (where "transfer" refers either to spatial or to chemical changes) are always directly proportional to the total amount of substance present in that pool. When a system is characterized by rates of transfer which are proportional to the  $n^{\text{th}}$  powers of the respective  $Q_i$ 's, it is still mathematically indistinguishable from a first order system, provided it is in dynamic equilibrium, i.e., equal amounts of the substance leave and enter each of its pools, and, therefore,  $Q_i$ 's are constant. Reactions with the rates of transfer proportional to powers of  $Q_i$ 's other than the first, occurring in systems not subject to equilibrium conditions<sup>(19,47,68)</sup>, are outside of the scope of this paper.

In the case of a general  $n$ -pool system (Fig. 1), where each pool empties into each of the remaining  $n - 1$  pools, the differential tracer equations of  $q_i$ 's are given by the following linear homogeneous system with constant coefficients:

$$\begin{aligned}\frac{dq_1}{dt} &= -k_1q_1 + A_{12}k_2q_2 + A_{13}k_3q_3 + \cdots + A_{1n}k_nq_n \\ \frac{dq_2}{dt} &= A_{21}k_1q_1 - k_2q_2 + A_{23}k_3q_3 + \cdots + A_{2n}k_nq_n \\ &\dots\dots\dots \\ \frac{dq_n}{dt} &= A_{n1}k_1q_1 + A_{n2}k_2q_2 + \cdots + A_{n(n-1)}k_{n-1}q_{n-1} - k_nq_n.\end{aligned}$$

Such a system of differential equations can be solved by means of Laplace transforms<sup>(9,10,26,60)</sup>.

When the  $n$  roots,  $-\lambda_1, -\lambda_2, \dots, -\lambda_n$  of the corresponding characteristic polynomial

$$D(p) = \begin{vmatrix} p + k_1 & -A_{12}k_2 & \cdots & -A_{1n}k_n \\ -A_{21}k_1 & p + k_2 & \cdots & -A_{2n}k_n \\ \dots\dots\dots \\ -A_{n1}k_1 & -A_{n2}k_2 & \cdots & p + k_n \end{vmatrix} = p^n + s_1p^{n-1} + \cdots + s_{n-1}p + s_n \\ = (p + \lambda_1)(p + \lambda_2) \cdots (p + \lambda_n) = 0$$

are all distinct, the solutions for  $q_i$ 's can be expressed as sums of  $n$  exponential functions:

$$q_i = q_i(t) = \alpha_{i1}e^{-\lambda_1 t} + \alpha_{i2}e^{-\lambda_2 t} + \cdots + \alpha_{in}e^{-\lambda_n t}$$

(When no ambiguity is possible,  $\alpha_{ij}$  may be replaced by  $\alpha_j$ .)

The initial conditions for the system are given by the set of numerical values  $q_{h0}$ 's;  $h = 1, 2, \dots, n$ . Substituting these values into the  $i^{\text{th}}$  column of the above determinant, replacing  $p$  by  $-\lambda_j$  and dividing by the product

$$\prod_{l \neq j} (-\lambda_j + \lambda_l)$$

for all  $l \neq j$ , gives the coefficient  $\alpha_{ij}$  of the  $j^{\text{th}}$  term of the solution of  $q_i$ . When the initial conditions are  $q_1(0) = q_0$ ;  $q_h(0) = 0$  for all  $h \neq 1$ , the solution becomes:

$$q_i = \sum_{j=1}^n \left\{ \frac{\begin{vmatrix} \cdots & -A_{1(i-1)}k_{i-1} & q_0 & -A_{1(i+1)}k_{i+1} & \cdots \\ \cdots & \cdots & \cdots & \cdots & \cdots \\ \cdots & -\lambda_j + k_{i-1} & 0 & -A_{i(i-1)}k_{i-1} & \cdots \\ \cdots & -A_{i(i-1)}k_{i-1} & 0 & -A_{i(i+1)}k_{i+1} & \cdots \\ \cdots & -A_{i(i+1)}k_{i+1} & 0 & -\lambda_j + k_{i+1} & \cdots \\ \cdots & \cdots & \cdots & \cdots & \cdots \end{vmatrix}}{\prod_{l \neq j} (-\lambda_j + \lambda_l)} \right\} e^{-\lambda_j t}$$



In the simplified notation, where  $N_i(p)$  refers to the determinant  $D(p)$  with the initial values substituted into its  $i^{\text{th}}$  column, and  $D'(p)$  stands for the derivative of  $D(p)$  with respect to  $p$ , the coefficients of the solution for  $q_i$  can be written as

$$\alpha_{ij} = \frac{N_i(-\lambda_j)}{D'(-\lambda_j)},$$

and therefore  $q_i$  becomes:

$$q_i = \sum_j \frac{N_i(-\lambda_j)}{D'(-\lambda_j)} e^{\lambda_j t} = \alpha_{i1} e^{\lambda_1 t} + \alpha_{i2} e^{\lambda_2 t} + \cdots + \alpha_{in} e^{\lambda_n t}$$

It should be noted that no restrictions have been imposed upon the values of the constants  $k_j$ 's and  $A_{ij}$ 's except that  $k_j$ 's are non-negative, and  $A_{ij}$ 's are both non-negative and satisfy the summation conditions

$$\sum_{i \neq j} A_{ij} \leq 1$$

for all  $j$ . These conditions, however, suffice to insure that all non-zero roots of  $D(p)$  have negative real parts<sup>(5)</sup>.

In writing the above solution for  $q_i$  it has been assumed that the characteristic polynomial  $D(p)$  had  $n$  distinct real zeros. In the case of  $m$  repeated roots:  $-\lambda_j = -\lambda_{j+1} = \cdots = -\lambda_{j+m-1}$ , the partial solution assumes the following form:<sup>(26)</sup>

$$\sum_{s=0}^{m-1} \left[ \frac{d^s}{dp^s} \left[ \frac{\begin{matrix} \cdots & -A_{1(i-1)}k_{i-1} & q_0 & -A_{1(i+1)}k_{i+1} & \cdots \\ \cdots & \cdots & \cdots & \cdots & \cdots \\ \cdots & -A_{n(i-1)}k_{i-1} & 0 & -A_{n(i+1)}k_{i+1} & \cdots \end{matrix}}{\prod_{l \neq j, \dots, j+m-1} (p + \lambda_l)} \right] \right] \frac{t^{m-s-1}}{s!(m-s-1)!} e^{\lambda_j t} \\ = \sum_{s=0}^{m-1} \left( \frac{d^s}{dp^s} \left[ \frac{(p + \lambda_j)^m N_i(p)}{D(p)} \right] \right) p = -\lambda_j \frac{t^{m-s-1}}{s!(m-s-1)!} e^{\lambda_j t}$$

The terms corresponding to each pair of complex roots can be rewritten as follows:

$$b_1 e^{-\lambda_R t - i\lambda_I t} + b_2 e^{-\lambda_R t + i\lambda_I t} = e^{-\lambda_R t} (b_1 e^{-i\lambda_I t} + b_2 e^{i\lambda_I t}) \\ = e^{-\lambda_R t} [B_1 \sin(\lambda_I t) + B_2 \cos(\lambda_I t)] = B e^{-\lambda_R t} \sin(\lambda_I t + \delta)$$

where  $\delta$  is a phase constant and where  $-\lambda_R$  and  $-\lambda_I$  are the real negative and the imaginary parts of the two conjugate roots respectively. When the  $\frac{dq_i}{dt}$ 's of the defining differential equations are linearly dependent upon  $m$  exponential functions  $e^{-\gamma_h t}$  (where  $\gamma_1, \gamma_2, \dots, \gamma_m$  are constants), as well as upon  $n$   $q_i$ 's, the corresponding solutions can still be easily obtained by means of Laplace transforms, although as many as  $m + n$  exponential functions may now appear in the expressions for  $q_i$ 's. In general, a set of  $n$  such defining equations can be replaced

by  $m + n$  linear homogeneous equations with constant coefficients, whose solutions can then be obtained as described above.

In the preceding, it has been indicated how starting with a general  $n$ -pool model, or rather with the  $n^2$  pool constants  $k_i$ 's and  $A_{ij}$ 's

$$\left( \sum_{i=1}^n A_{ij} \leq 1 \right),$$

we can obtain the functions  $q_i(t)$  of the tracer distribution within each of the  $n$  pools  $Q_i$ . In practice, however, we are more concerned with the converse problem, namely that of determining the pool constants from the knowledge of one or more tracer distribution equations,  $q_i$ , i.e., the approximating equations to the experimentally observed behavior of a tracer in one or more pools,  $Q_i$ , which are accessible to sampling. In considering an approximating equation for a single accessible pool, it is necessary to decide upon the number of pools involved in the immediate system. It will be assumed that if (in the absence of any special information) experimental data can be adequately represented by a sum of  $n$  exponential functions, then  $n$  is the required number of pools; this is equivalent to postulating that all  $e^{-\lambda_i t}$ 's, where  $-\lambda_1, \dots, -\lambda_n$  are  $n$  distinct roots of an  $n^{\text{th}}$  degree characteristic equation  $D(p)$ , have non-vanishing coefficients. Although this decision may at first appear arbitrary, it is usually the only logical one insofar as it is based exclusively upon the information which is experimentally available, and permits us to deal with systems of minimal complexity. As an example, consider the so-called mammillary system which consists of a central pool exchanging with  $m$  peripheral mutually non-interchanging pools<sup>(52)</sup>. For a large  $m$ , the experimental tracer data derived from such a general system would not in practice be amenable to a rigorous mathematical analysis in terms of an  $(m + 1)$ -pool model. Under certain rather broad assumptions, however, such a system behaves as a simple two-pool system. Assuming that all peripheral pools possess the same relative rate  $k$ , it can be shown that the tracer equation of the central pool of such a system with  $m = 2$  (and hence by mathematical induction with any  $m \geq 2$ ) is identical with the corresponding function of an appropriate two-pool system. When the lateral pools of the mammillary system are allowed to interchange, the now more complicated system, can still behave as a two-pool model under additional assumptions about the interchange constants between lateral pools<sup>(52)</sup>. It is precisely this type of reasoning which justifies the approximation of such immensely complicated systems as the interchange of iron or sodium between plasma and the remaining extracellular space in man, by relatively simple two- or three-pool models.

It is expedient at this point to mention a very different type of approximation, which can be applied to a fairly long series of unilaterally connected pools, i.e., to an ordered sequence of pools in which the transfer of the basic substance takes place only in one direction from the  $i^{\text{th}}$  to the  $(i + 1)^{\text{st}}$  pool<sup>(53)</sup>. Such a system, which may represent a chain of irreversible chemical reactions, can be approximated by an infinite series. Let the total amount,  $Q$ , of the basic substance in

the system remain constant as the number  $n$  of the individual vanishingly small pools of the series approaches infinity. Assume that a tracer is introduced into the system somewhere prior to the beginning of the series; then, if this series empties into an accessible terminal pool,  $Q_T$ , with a relative rate,  $k_s$  (where  $k_s$  is defined as the amount transferred from the last pool of the series into  $Q_T$  per unit time, divided by the total amount  $Q_s$ ), its presence can be detected in the tracer distribution of  $Q_T$  only as a time lag of duration  $\frac{1}{k_s}^{(53)}$ . As examples of

cases where such approximations may be applicable in the study of metabolic processes in man, consider 1) the time lag between the introduction of  $\text{Fe}^{59}$  into the bone marrow and its appearance in the hemoglobin of the circulating erythrocytes, and 2) the time lag between the incorporation of  $\text{I}^{131}$  into the thyroid gland and the subsequent appearance of the  $\text{I}^{131}$  labelled hormone in plasma. (The general problem of pool systems with a time lag, that is, systems where the tracer recycles (circulates repeatedly through a gap), will be dealt with in a subsequent publication.)

In view of the above discussion, it will be assumed that the number  $n$  of distinct exponential functions in the tracer equation of an accessible pool is equal to the number of pools in the system. We will further restrict ourselves (unless otherwise specified) to the consideration of "reduced" systems, i.e., systems where for each  $i$  and  $j$ , the tracer material introduced into the  $i^{\text{th}}$  pool moves from the  $i^{\text{th}}$  to the  $j^{\text{th}}$  pool, either directly or by passing through a series of intermediary pools. "Reduced" system refers only to that part of a total pool system in which bilateral (though not necessarily direct) interchange exists between any two component pools. Pools which do not thus interchange with the "reduced" part of the system (unidirectionally connected pools, or "accumulation pools"), do not influence the tracer behavior of the reduced system, and therefore, do not affect the number of its constraints and degrees of freedom (see below). Such reduced systems can be characterized by the existence of at least one chain-constant  $A_{ik}A_{kl} \cdots A_{mh}A_{hj} \neq 0$  for each  $i$  and  $j$ . Before considering the actual evaluations of pool-constants from a given set of solutions of  $q_i$ 's, it is desirable to obtain a general formulation of 1) the number of pool-constants (or equivalently of their independent functions) which completely determine a reduced  $n$ -pool system, 2) the number of such independent functions (or constraints) which can be obtained from the knowledge of all or some of the solutions of the  $q_i$ 's and hence 3) the number of the degrees of freedom, i.e., the number of additional conditions or constraints which can be further imposed upon the system under consideration.  $n + 2$  constants can be directly associated with each of the pools  $Q_i$  of a reduced system in dynamic equilibrium, namely  $\dot{Q}_i$ ,  $S_i$ ,  $E_i = A_{0,i}k_iQ_i$  and  $A_{j,i}k_iQ_i$ 's for all  $j \neq i$ . (Some of the  $A_{j,i}$ 's may be zeros.) Hence the total number of such defining constants is given by  $n(n + 2)$ . Note that the assumption of dynamic equilibrium is implicit in the above considerations insofar as quantities  $Q_i$ ,  $E_i$ , etc. are utilized. Figure 2 illustrates the case of  $n = 3$ . Only  $n^2$  of the pool constants (namely  $k_i$ 's and  $A_{ij}$ 's;  $i, j = 1, \cdots, n$ ;  $i \neq j$ ) appear in the differential equations describing the state of the system, and hence

in their solutions. The  $n$  solutions,  $q_i$ 's, yield  $n^2$  functions of these pool constants. The  $n$  exponents,  $-\lambda_i$ , form  $n$  symmetric functions,  $s_i$  (where  $s_1 = \sum \lambda_i$ ,  $s_2 = \frac{1}{2} \sum \lambda_i \lambda_j$ ,  $\dots$ , etc.) which in turn are functions of  $k_i$ 's and  $A_{ij}$ 's (see above).

In addition, each of the  $n$  sets of the coefficients  $\alpha_{ij}$  ( $j = 1, \dots, n$ ) consists of  $n - 1$  independent functions of  $\lambda_i$ 's,  $A_{ij}$ 's and  $k_i$ 's, that is of  $k_i$ 's and  $A_{ij}$ 's only. (Note that each complete set of  $n$   $\alpha_{ij}$ 's,  $j = 1, \dots, n$ , satisfies also the relation describing the initial condition for  $q_i(0)$ .) Therefore, when all the solutions of

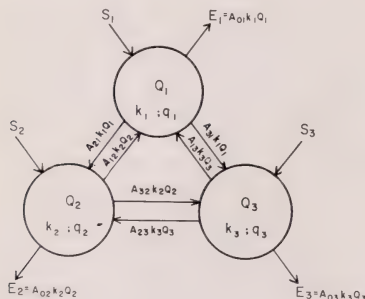


FIG. 2. Schematic representation of a three-pool reduced system demonstrating how five (i.e.,  $n + 2$ ) constants are associated with each pool.

TABLE I  
*Reduced  $n$ -pool Systems in Dynamic Equilibrium, with One Accessible Pool*

Number of pools	Number of constants determining the system	Number of constraints (including $Q_i$ and $n$ equilibrium conditions)	Number of degrees of freedom
$n$	$n(n + 2)$	$3n$	$n(n - 1)$
1	3	3	0
2	8	6	2
3	15	9	6
...	...	...	...

the tracer equations are known, i.e.,  $n^2$  independent functions or constraints of the  $n^2$  pool constants  $k_i$ 's and  $A_{ij}$ 's are available, these constants are uniquely determined. When in addition  $n$  equilibrium conditions are imposed upon the system, only  $n$  degrees of freedom remain:  $n(n + 2) - n^2 - n = n$ . These may be either  $n$   $Q_i$ 's,  $n$   $S_i$ 's,  $n$   $E_i$ 's, or in general  $n$  independent functions of  $Q_i$ 's,  $S_i$ 's, and  $E_i$ 's. In most practical instances, however, only one pool, say  $Q_1$ , is accessible and, therefore,  $2n$  constraints (besides the  $n$  equilibrium conditions) must be satisfied; these include the value of  $Q_1$  itself, as well as  $n$   $\lambda_i$ 's and  $n - 1$  of the  $n$  coefficients  $\alpha_{ij}$  of the solution  $q_1 = \alpha_{11}e^{-\lambda_1 t} + \alpha_{12}e^{-\lambda_2 t} + \dots + \alpha_{1n}e^{-\lambda_n t}$ . The case of a reduced  $n$ -pool system with only one accessible pool is summarized in Table I.

If we consider the pool-system from the point of view of tracer distribution only where the tracer is subject to the first order reaction rates, the equilibrium conditions are no longer relevant and the actual amounts of the basic substance present in, leaving, or entering each pool, do not affect the distribution of the tracer. In fact, the basic substance need not be present in the organism at all, as may be the case in the distribution studies of non-physiological substances. In such cases, Table I should be replaced by Table II.

In conclusion, it should be noted that in the general case of a reduced system, the coefficients  $\alpha_{ij}$  of the exponential function  $e^{-\lambda_j t}$  with the smallest real  $\lambda_j$  are positive,  $\alpha_{ij} > 0$ , for all  $i$ . After a sufficiently long time interval,  $t$ , the  $\alpha_{ij}e^{-\lambda_j t}$  for such smallest real  $\lambda_j$  will become the dominant term of each solution,  $q_i$ . Eventually these solutions will approach constant ratios and can be represented by parallel lines on semilog paper.

TABLE II  
*Reduced n-pool Tracer Distribution Systems*

Number of pools	Number of accessible pools	Number of constants determining the system	Number of available functions (not including $Q_i$ 's and equilibrium conditions)	Number of degrees of freedom
$n$	$m$	$n^2$	$n + m(n - 1)$	$(n - 1)(n - m)$
1	1	1	1	0
2	1	4	3	1
2	2	4	4	0
3	1	9	5	4

## TWO-POOL SYSTEMS WITH SPECIAL EMPHASIS ON MODELS FOR IRON METABOLISM

### *Section 1. Solutions of two-pool systems.*

In the case of a two-pool system (Fig. 3), the equations and solutions discussed above reduce to the following:

$$\frac{dq_1}{dt} = -k_1q_1 + A_{12}k_2q_2$$

$$\frac{dq_2}{dt} = A_{21}k_1q_1 - k_2q_2$$

$$D(p) = \begin{vmatrix} p + k_1 & -A_{12}k_2 \\ -A_{21}k_1 & p + k_2 \end{vmatrix} = (p + k_1)(p + k_2) - A_{12}A_{21}k_1k_2$$

$$= p^2 + (k_1 + k_2)p + (1 - A_{12}A_{21})k_1k_2 = (p + \lambda_1)(p + \lambda_2).$$

Let  $-\lambda_1$  and  $-\lambda_2$  be the roots of  $D(p) = 0$ : Then,

$$\lambda_1 = \frac{k_1 + k_2 + \sqrt{(k_1 + k_2)^2 - 4(1 - A_{12}A_{21})k_1k_2}}{2}$$

$$\lambda_2 = \frac{k_1 + k_2 - \sqrt{(k_1 + k_2)^2 - 4(1 - A_{12}A_{21})k_1k_2}}{2}.$$

The derivative of  $D(p)$  with respect to  $p$  becomes

$$D'(p) = 2p + k_1 + k_2.$$

The initial conditions are given as  $q_1(0) = q_0$  and  $q_2(0) = 0$ . Hence

$$N_1(p) = \begin{vmatrix} q_0 & -A_{12}k_2 \\ 0 & p + k_2 \end{vmatrix} = q_0(p + k_2)$$

$$N_2(p) = \begin{vmatrix} p + k_1 & q_0 \\ -A_{21}k_1 & 0 \end{vmatrix} = q_0 A_{21}k_1.$$

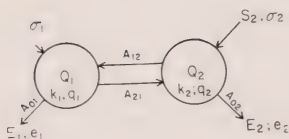


FIG. 3. Schematic representation of a two-pool system.

From this it follows that, for  $-\lambda_1 \neq -\lambda_2$ ,

$$\begin{aligned} \frac{q_1}{q_0} &= \frac{N_1(-\lambda_1)}{D'(-\lambda_1)} e^{-\lambda_1 t} + \frac{N_1(-\lambda_2)}{D'(-\lambda_2)} e^{-\lambda_2 t} \\ &= \sqrt{\frac{-(k_1 + k_2)}{2\lambda}} e^{-\lambda_1 t} + \sqrt{\frac{-(k_1 + k_2)}{2\lambda}} e^{-\lambda_2 t} \end{aligned}$$

and

$$\frac{q_2}{q_0} = \frac{N_2(-\lambda_1)}{D'(-\lambda_1)} e^{-\lambda_1 t} + \frac{N_2(-\lambda_2)}{D'(-\lambda_2)} e^{-\lambda_2 t} = \frac{A_{21}k_1}{\lambda} (e^{-\lambda_1 t} - e^{-\lambda_2 t}),$$

where  $\lambda$ 's are given above, and  $\sqrt{\quad}$  stands for

$$\sqrt{(k_1 + k_2)^2 - 4(1 - A_{12}A_{21})k_1k_2}.$$

When  $-\lambda_1 = -\lambda_2$ , the discriminant of the characteristic polynomial  $D(p)$  is equal to zero,  $(k_1 + k_2)^2 - 4(1 - A_{12}A_{21})k_1k_2 = 0$ , and therefore both  $A_{12}A_{21} = 0$  and  $k_1 = k_2$ . Since  $A_{21} = 0$  implies a one-pool system, we only need to consider the special case of a two-pool system with unidirectional flow, i.e., with  $A_{12} = 0$ , and with equal relative rates  $k_1 = k_2 = k$ :

$$\frac{dq_1}{dt} = -kq_1$$

$$\frac{dq_2}{dt} = A_{21}kq_1 - kq_2$$

The solutions for the repeated roots  $-\lambda_1 = -\lambda_2$  become:



$$\frac{q_1}{q_0} = e^{-k_1 t}$$

$$\frac{q_2}{q_0} = A_{21} k_1 e^{-k_1 t}$$

(see Appendix for an alternate proof).

In the case of unidirectional flow with unequal relative rates (i.e.,  $A_{12} = 0$  and  $k_1 \neq k_2$ ), the zeros of  $D(p)$  become  $-\lambda_1 = -k_1$  and  $-\lambda_2 = -k_2$ , and therefore:

$$\frac{q_1}{q_0} = e^{-k_1 t}$$

$$\frac{q_2}{q_0} = \frac{A_{21} k_1}{k_1 - k_2} (e^{-k_2 t} - e^{-k_1 t}).$$

Finally, in the case of a closed system, i.e., a system for which the basic substance neither enters nor leaves the system as a whole ( $A_{12} = A_{21} = 1$  and  $A_{01} = A_{02} = 0$ ), the two zeros (i.e., solutions) of the characteristic equation  $D(p)$  become

$$-\lambda_1 = -(k_1 + k_2) \quad \text{and} \quad -\lambda_2 = 0,$$

and therefore the two solutions are:

$$\frac{q_1}{q_0} = \frac{k_1}{k_1 + k_2} e^{-(k_1 + k_2)t} + \frac{k_2}{k_1 + k_2} = \frac{k_2}{k_1 + k_2} \left( 1 + \frac{k_1}{k_2} e^{-(k_1 + k_2)t} \right)$$

and

$$\frac{q_2}{q_0} = \frac{k_1}{k_1 + k_2} (1 - e^{-(k_1 + k_2)t}).$$

It should be noted that when the two pools,  $Q_1$  and  $Q_2$ , are spatially indistinguishable, the total tracer content  $q_T$  of the combined pool  $Q_T = Q_1 + Q_2$  is given by  $q_T = q_1 + q_2$ . If the initial tracer dose  $q_0$  is introduced into  $Q_1$  exclusively, the equation for  $q_T$  becomes

$$\frac{q_T}{q_0} = \frac{\sqrt{-} + (k_1 - k_2) - 2A_{21}k_1}{2\sqrt{-}} e^{-\lambda_1 t} + \frac{\sqrt{-} - (k_1 - k_2) + 2A_{21}k_1}{2\sqrt{-}} e^{-\lambda_2 t}.$$

If only a fraction of the initial tracer dose  $q_0$  is introduced into  $Q_1$  (and the remainder into  $Q_2$ ), the expression for  $\frac{q_T}{q_0}$  becomes more complicated and will be discussed in Section 4.

In the present section the tracer equations (as functions of time) for given two-pool systems, i.e., for given sets of parameters have been considered. In practice, the more important problem is the converse, namely that of determining the pool constants from derived tracer equations. From Table I it follows

that a single such equation does not suffice to determine completely a two-pool system. Even when the system is assumed to be in dynamic equilibrium, and when in addition to the constants of the tracer equation, the amount of the stable isotope in the accessible pool is known, two degrees of freedom still remain. Therefore a general steady state two-pool system with a single accessible pool is indeterminate, unless two additional independent conditions are imposed upon it. Such pairs of conditions may assume a variety of forms which, however, can be shown to be equivalent to each other. Since we are primarily interested in iron metabolism, we will in the following section express these conditions in terms most appropriate for this purpose, rather than seek the simplest or most elegant formulation of the problem.

*Section 2. Approximation of two-pool tracer distribution data (plasma  $\text{Fe}^{59}$  disappearance) by a sum of two exponential functions.*

In Section 1, pool systems were considered from the theoretical viewpoint only, without direct reference to their physiological counterparts. An analysis of some aspects of iron kinetics as reflected in the disappearance of  $\text{Fe}^{59}$  from plasma during the first four to six hours after intravenous injection will now be considered<sup>51</sup>. The results of this analysis will be applicable to other similar systems where the tracer disappearance data from an accessible pool, or a combination of pools, can be reasonably well approximated, over a sufficiently long period of time, by a sum of two exponential functions:  $q_1(t) = \alpha_1 e^{-\lambda_1 t} + \alpha_2 e^{-\lambda_2 t}$  (here  $\alpha_1$  is replaced by  $\alpha_1$  and  $\alpha_2$  by  $\alpha_2$ ). Such approximation or curve-fitting can be achieved in any one of the several accepted ways, including the use of an analogue computer.

In the case of plasma  $\text{Fe}^{59}$  disappearance data,  $\lambda_1$  is usually significantly larger than  $\lambda_2$ , and after two to four hours the value of  $\alpha_1 e^{-\lambda_1 t}$  becomes small in comparison with that of  $\alpha_2 e^{-\lambda_2 t}$ . Therefore, in practice, the curve-fitting may proceed as follows: drawing the approximating straight line  $y_2 = \alpha_2 e^{-\lambda_2 t}$  (whenever feasible this is drawn through the last few experimental points plotted on semilog paper<sup>5</sup>), and subtracting from the given data the ordinates of the corresponding points on the above line, will give a new set of points which must then in turn be approximated by another straight line  $y_1 = \alpha_1 e^{-\lambda_1 t}$  on the semilog paper. Sometimes several changes in the assumed function  $\alpha_2 e^{-\lambda_2 t}$  are necessary before a reasonably good fit  $\alpha_1 e^{-\lambda_1 t}$  can be obtained. (This is especially true when  $\alpha_1 e^{-\lambda_1 t}$  does not become sufficiently small for  $t \leq 4$  hours.) The sum of the above two exponential functions then forms the first rough approximation. Further refinements are achieved by slightly varying the values of  $\lambda_1$  and  $\lambda_2$ , and by determining numerically by trial and error the most suitable values for  $\alpha_1$  and  $\alpha_2$  for each set of values of  $\lambda_1$  and  $\lambda_2$ . The final theoretical values, as well as the experimental points are then normalized by dividing them by the initial value of the tracer  $q_0 = \alpha_1 + \alpha_2$ . The initial values  $q(0) = q_0$ , as well as  $q(t) = \alpha_1 e^{-\lambda_1 t} + \alpha_2 e^{-\lambda_2 t}$ , represent either the total amount of tracer present in a given pool at the time  $t$ , or an aliquot thereof. These values can be expressed in terms of milligrams, millicuries, or as counts per unit time per given volume. The normalized values

$\frac{q(t)}{q_0}$ , therefore, represent the fractional amounts of the initial dose remaining in plasma at the time  $t$ .

It should be emphasized that as long as the experimental plasma  $\text{Fe}^{59}$  disappearance data of the first four to six hours are closely approximated by the sum of two exponential functions (i.e., within the accuracy determined by the standard deviation inherent in the measuring and counting techniques), it is neither necessary nor justifiable to assume more than two pools with significant interchange during the initial experimental period. This is not inconsistent with the assumption of a more complicated pool system when considered over a longer period of time. The pool constants derived from a long range analysis will not differ significantly from the corresponding constants calculated on the basis of the initial two pool system.

The mere fact that plasma  $\text{Fe}^{59}$  disappearance data can be closely approximated by a sum of two exponential functions, opens the possibility of three alternative fundamental assumptions:

- I. Plasma iron represents one of two spatially separated interchanging pools.
- II. Plasma iron consists of two spatially indistinguishable interchanging pools which differ from each other in chemical state or in reactivity of their iron.
- III. Plasma iron consists of two spatially indistinguishable non-interchanging iron pools.

*Section 3. Plasma iron as one of two spatially separated interchanging pools of iron metabolism.*

From the practical point of view, this is probably the most important alternative. Here plasma represents the accessible pool  $Q_1$  with the known quantity  $Q_1$  of the stable isotope. The three constants  $k_1$ ,  $k_2$  and  $A_{12}A_{21}$  can be obtained from the normalized approximating equation

$$\frac{q_1}{q_0} = a_1 e^{-\lambda_1 t} + a_2 e^{-\lambda_2 t}$$

(where,

$$a_1 = \frac{\sqrt{-} + (k_1 - k_2)}{2\sqrt{-}}$$

and

$$a_2 = \frac{\sqrt{-} - (k_1 - k_2)}{2\sqrt{-}},$$

are normalized coefficients of the exponential functions, and where,

$$\lambda_1 = \frac{k_1 + k_2 + \sqrt{-}}{2},$$

$$\lambda_2 = \frac{k_1 + k_2 - \sqrt{-}}{2},$$

and

$$\sqrt{-} = \sqrt{(k_1 + k_2)^2 - 4(1 - A_{12}A_{21})k_1k_2} \quad )$$

as follows:

$$k_1 + k_2 = \lambda_1 + \lambda_2$$

and

$$k_1 - k_2 = (a_1 - a_2) \sqrt{-} = (a_1 - a_2)(\lambda_1 - \lambda_2)$$

yield the values of  $k_1$  and  $k_2$  in terms of  $a_1$ ,  $a_2$ ,  $\lambda_1$  and  $\lambda_2$ . The value of  $A_{12}A_{21}$  follows then from the expression

$$1 - A_{12}A_{21} = \frac{\lambda_1\lambda_2}{k_1k_2}.$$

According to Table I, this leaves two degrees of freedom in cases of dynamic equilibrium. The two conditions which will now be imposed upon the system and which must not be functions of  $Q_1$ ,  $k_1$ ,  $k_2$  and  $A_{12}A_{21}$  alone, can be given as

$$\sigma_1 = \frac{S_1}{S_1 + S_2} \quad \left( \text{or } \sigma_2 = 1 - \sigma_1 = \frac{S_2}{S_1 + S_2} \right),$$

and

$$\omega_1 = \frac{E_1}{E_1 + E_2} \quad \left( \text{or } \omega_2 = 1 - \omega_1 = \frac{E_2}{E_1 + E_2} \right)$$

and will be referred to as the modes of entry and exit of the basic substance into and from the two-pool system. In the following we will limit ourselves to only four modes of entry and exit respectively, which will result in sixteen possible combinations, i.e., in sixteen different models of the general two-pool system. Below, in the definitions of the four modes of exit and of entry considered, the notations in parentheses refer to modes of entry:

I (or a): All exit from (or entry to) the system takes place through pool I, i.e., the accessible pool  $Q_1$ .

II (or b): All exit from (or entry to) the system takes place through pool II.

III (or c): Fraction  $U$ , of the total amount leaving (or entering) the system, leaves (or enters) through pool I; fraction  $1 - U$ , leaves (or enters) through pool II. Here  $U_e$ , the effective uptake, is defined as

$$U_e = \frac{\text{amt. of Fe leaving the system which is utilized for Hgb. synthesis}}{\text{total amount of iron leaving the (two-pool) system}}$$

This should be distinguished from the maximum uptake,  $U$ , which is the fraction of initial tracer dose  $q_0$  utilized for hemoglobin formation.

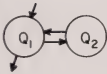
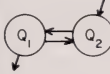
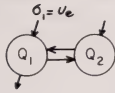
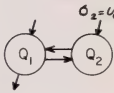
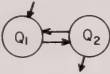
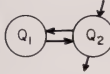
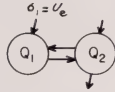
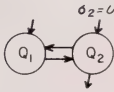
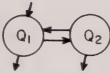
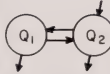
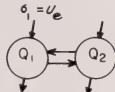
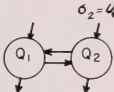
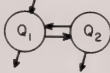
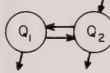
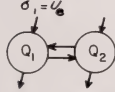
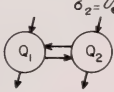
IV (or d): Fraction  $1 - U_e$  of the total amount leaving (or entering) the system, leaves (or enters) through pool I; fraction  $U_e$ , leaves (or enters) through pool II.

In Table III the 16 models are grouped according to their modes of exit and entry which in turn can be equally well described in terms of  $e_1$ , i.e., the fraction of initial tracer dose  $q_0$  which eventually leaves the system through pool I; in

TABLE III

*Sixteen Extreme Two-Pool Models Characterized by Their Modes of Exit and Entry*

## MODE OF ENTRY

	ALL ENTRY THROUGH POOL I	ALL ENTRY THROUGH POOL II	FRACTION $\sigma_1 = U_e$ OF TOTAL AMOUNT ENTER- ING THE SYSTEM ENTERS THROUGH POOL I FRACTION $\sigma_2 = 1 - U_e$ ENTERS POOL II	FRACTION $\sigma_1 = 1 - U_e$ ENTERS THROUGH POOL I, FRACTION $\sigma_2 = U_e$ ENTERS POOL II
GROUP I ALL EXIT THROUGH POOL I ( $e_1 = 1$ )	 Ia	 Ib	 Ic	 Id
GROUP II ALL EXIT THROUGH POOL II ( $e_1 = 0$ )	 IIa	 IIb	 IIc	 IIc
GROUP III FRACTION $\omega_1 = U_e$ OF TOTAL AMOUNT LEAVING THE SYSTEM LEAVES THROUGH POOL I FRACTION $\omega_2 = 1 - U_e$ LEAVES POOL II ( $e_1 = U$ )	 IIIa	 IIIb	 IIIc	 IIId
GROUP IV FRACTION $\omega_1 = 1 - U_e$ LEAVES THROUGH POOL I FRACTION $\omega_2 = U_e$ LEAVES POOL II ( $e_1 = 1 - U$ )	 IVa	 IVb	 IVc	 IVd

group I,  $e_1 = 1$ ; in group II,  $e_1 = 0$ ; in group III,  $e_1 = U$ ; and finally in group IV,  $e_1 = 1 - U$ .

Each of the sets of two defining relations (mode of entry and mode of exit) imposed upon the above models, together with the constants of the approxim-

ing equation for the plasma  $\text{Fe}^{59}$  disappearance, the equilibrium conditions, and the total plasma iron,  $Q_1$ , determine a two-pool system completely. The parameters which are of particular physiological significance, and which can now be calculated for each of the sixteen models, are the following:

1.  $Q_2$  = quantity of iron in the second pool,  $Q_2$ .
2.  $R_e = E_1 - E_2$  = the effective iron turnover, i.e., the absolute rate of disappearance of iron from the two-pool system as a whole.
3.  $U_e$  = effective uptake, i.e., fraction of  $R_e$  which is utilized for the formation of hemoglobin.

In studying iron metabolism it will be convenient to express  $A_{01}$  and  $A_{02}$  (and hence also,  $A_{12}$  and  $A_{21}$ ) as explicit functions of  $e_1$ , i.e., fractions of tracer leaving the two-pool system through plasma,  $Q_1$ , and the interchange constant  $A_{12}A_{21}$ . From the definitions of  $e_1$  and  $E_1 = A_{01}k_1q_1$ , it follows that

$$e_1 = \frac{1}{q_0} \int_0^\infty A_{01}k_1q_1 dt = A_{01}k_1 \int_0^\infty (a_1e^{-\lambda_1 t} + a_2e^{-\lambda_2 t}) dt = A_{01}k_1 \left( \frac{a_1}{\lambda_1} + \frac{a_2}{\lambda_2} \right),$$

and

$$e_2 = 1 - e_1 = \frac{A_{02}A_{21}k_1k_2}{\lambda_1\lambda_2} \int_0^\infty (e^{-\lambda_2 t} - e^{-\lambda_1 t}) dt$$

Substituting the corresponding pool constants for  $a_1$ ,  $a_2$ ,  $\lambda_1$  and  $\lambda_2$  we get

$$\begin{aligned} e_1 &= \frac{A_{01}}{1 - A_{12}A_{21}} \\ A_{01} &= e_1(1 - A_{12}A_{21}) \\ A_{02} &= \frac{(1 - e_1)(1 - A_{12}A_{21})}{1 - e_1(1 - A_{12}A_{21})} \\ A_{21} &= 1 - e_1(1 - A_{12}A_{21}) \\ A_{12} &= \frac{A_{12}A_{21}}{1 - e_1(1 - A_{12}A_{21})} \end{aligned}$$

In the following, the three equilibrium conditions ("E.C.'s"), only two of which are independent, will be referred to by their numbers as follows:

E.C. I stands for  $S_1 + S_2 = E_1 + E_2 = A_{01}k_1Q_1 + A_{02}k_2Q_2$ .

E.C. II stands for  $S_1 + A_{12}k_2Q_2 = E_1 + A_{21}k_1Q_1 = k_1Q_1$ .

E.C. III stands for  $S_2 + A_{21}k_1Q_1 = E_2 + A_{12}k_2Q_2 = k_2Q_2$ .

*Physiological constants for the models of group I.*

In this case all exit takes place through pool  $Q_1$ , which implies,

$$E_2 = 0; e_1 = 1; A_{02} = 0; A_{12} = 1; U_e = U.$$

Hence



$$A_{21} = A_{12}A_{21}$$

$$A_{01} = 1 - A_{21} = 1 - A_{12}A_{21}$$

and, therefore,

$$R_e = E_1 + E_2 = A_{01}k_1Q_1 = (1 - A_{12}A_{21})k_1Q_1.$$

The amount  $Q_2$  of the second pool must be calculated separately for each model in this group. For instance in the case of Model Ic we get the relation  $\sigma_1 = U_e = U$  and hence  $S_1 = \sigma_1(S_1 + S_2) = \sigma_1E_1 = UA_{01}k_1Q_1$ . Substituting this last value for  $S_1$  in E.C. II, we get

$$k_1Q_1 = S_1 + A_{12}k_2Q_2 = UA_{01}k_1Q_1 + k_2Q_2$$

or

$$Q_2 = [1 - U(1 - A_{12}A_{21})] \frac{k_1}{k_2} Q_1.$$

*Physiological constants for the models of group III.*

In this group  $e_1 = U$ . Therefore,  $A_{01}$  and  $A_{02}$  can be written as

$$A_{01} = e_1(1 - A_{12}A_{21}) = U(1 - A_{12}A_{21})$$

$$A_{02} = \frac{(1 - U)(1 - A_{12}A_{21})}{1 - U(1 - A_{12}A_{21})}.$$

Substituting these values for  $A_{01}$  and  $A_{02}$  into the equation

$$R_e = A_{01}k_1Q_1 + A_{02}k_2Q_2$$

we get

$$R_e = U(1 - A_{12}A_{21})k_1Q_1 + \frac{(1 - U)(1 - A_{12}A_{21})}{1 - U(1 - A_{12}A_{21})} k_2Q_2.$$

Similarly,

$$U_e = \frac{E_1}{E_1 + E_2} = \frac{A_{01}k_1Q_1}{R_e} = \frac{U(1 - A_{12}A_{21})k_1Q_1}{R_e}.$$

In the case of Model IIIc there exists an additional relation  $S_1 = E_1$ , and E.C. II reduces to  $A_{12}k_2Q_2 = A_{21}k_1Q_1$ . Hence

$$Q_2 = \frac{A_{21}k_1}{A_{12}k_2} Q_1 = \frac{[1 - U(1 - A_{12}A_{21})]^2 k_1}{A_{12}A_{21}} Q_1,$$

$$R_e = \frac{A_{12}A_{21} + (1 - U)^2(1 - A_{12}A_{21})}{A_{12}A_{21}} (1 - A_{12}A_{21})k_1Q_1.$$

Table IV gives the values of  $Q_2$ ,  $R_e$  and  $U_e$  in terms of  $k_1$ ,  $k_2$ ,  $A_{12}A_{21}$ ,  $Q_1$  and  $U$  for each of the sixteen models.

The above choice of sixteen representative models was governed by the as-

TABLE IV  
*Calculations of Clinical Parameters of Iron Metabolism for the Sixteen Extreme Two-Pool Models*

Factor	Models whose values for $Q_2$ are obtained by multiplying the corresponding factor by $Q_0 = \frac{k_1}{k_2} Q_1$ .	Models whose values for $R_2$ are obtained by multiplying the corresponding factor by $R_0 = (1 - A_{12}A_{21})k_1Q_1$	Models whose values for $U_r$ are obtained by multiplying the corresponding factor by $U$	Models whose values for $U_r R_c$ are obtained by multiplying the corresponding factor by $R_0 U = (1 - A_{12}A_{21})k_1Q_1U$
1	Ib; IIa; IIId; IVc.	Ia, b, c, d; IIa; IIIa; IVa.	Ia, b, c, d; IIa, b, c, d; IIIa; IVa.	Ia, b, c, d; IIa; IIIa, b, c, d; IVa.
$A_{12}A_{21}$	Ia			
$\frac{1}{A_{12}A_{21}}$	IIb	IIb		IIb; IVb
$1 - U(1 - A_{12}A_{21})$	Ic; IIIa			
$\frac{1}{1 - U(1 - A_{12}A_{21})}$	IIId	IIId		IIId
$\frac{1 - U(1 - A_{12}A_{21})}{A_{12}A_{21}}$	IIIb	IIIb		
$\frac{A_{12}A_{21}}{1 - U(1 - A_{12}A_{21})}$			IIIb	
$A_{12}A_{21} + U(1 - A_{12}A_{21})$	Id; IVa			
$\frac{1}{A_{12}A_{21} + U(1 - A_{12}A_{21})}$	IIc	IIc	IVb	IIc; IVc
$\frac{A_{12}A_{21} + U(1 - A_{12}A_{21})}{A_{12}A_{21}}$	IVb	IVb		IVd
$\frac{[A_{12}A_{21} + U(1 - A_{12}A_{21})]^2}{A_{12}A_{21}}$	IVd			
$\frac{[1 - U(1 - A_{12}A_{21})]^2}{A_{12}A_{21}}$	IIIc			
$\frac{1 - U(1 - A_{12}A_{21})}{1 - U^2(1 - A_{12}A_{21})}$			IIId	
$\frac{A_{12}A_{21} + (1 - U)^2(1 - A_{12}A_{21})}{A_{12}A_{21}}$		IIIc		
$\frac{A_{12}A_{21}}{A_{12}A_{21} + (1 - U)^2(1 - A_{12}A_{21})}$			IIIc	

TABLE IV—*Continued*

Factor	Models whose values for $Q_2$ are obtained by multiplying the corresponding factor by $Q_0 = \frac{k_1}{k_2} Q_1$ .	Models whose values for $R_e$ are obtained by multiplying the corresponding factor by $R_0 = (1 - A_{12}A_{21})/k_1Q_1$	Models whose values for $U_e$ are obtained by multiplying the corresponding factor by $U$	Models whose values for $U_e R_e$ are obtained by multiplying the corresponding factor by $R_0 U = (1 - A_{12}A_{21})/k_1Q_1 U$
$\frac{1 - U^2(1 - A_{12}A_{21})}{1 - U(1 - A_{12}A_{21})}$		III d		
$\frac{1 - (1 - U)^2(1 - A_{12}A_{21})}{A_{12}A_{21} + U(1 - A_{12}A_{21})}$		IV c		
$\frac{1}{1 - (1 - U)^2(1 - A_{12}A_{21})}$			IV c	
$\frac{A_{12}A_{21} + U^2(1 - A_{12}A_{21})}{A_{12}A_{21}}$		IV d		
$\frac{A_{12}A_{21} + U(1 - A_{12}A_{21})}{A_{12}A_{21} + U^2(1 - A_{12}A_{21})}$			IV d	

sumption that all iron destined for red cell formation leaves the system through only one of the two pools, and that the remaining iron leaves either through the same pool or through the other pool only. Similar assumptions were made about the iron entering the system. It should be emphasized that this particular selection of models is arbitrary, and that none of the models may reflect the actual mechanism of iron metabolism. Though theoretically  $\sigma_i$  and  $\omega_i$  can assume all values between zero and one ( $0 \leq \sigma_i, \omega_i \leq 1$ ), in practice we must start by considering only a limited number of physiologically feasible models. It should be possible to reduce the number of such initial models by means of a detailed analysis of different observed physiological parameters in cases of blood dyscrasias and in normal subjects. Some of the models may yield data inconsistent with those obtained by other proven methods, or contradictory to well established physiological principles. It is this type of reasoning applied to hemoglobin renewal in polycythemia vera that allows for elimination of Models IIb, II d, IVb and IVd<sup>(51)</sup>. Similarly the elimination of all Models of group I (Ia, Ib, Ic, Id) follows from *in vivo* studies<sup>(54)</sup>.

Among the parameters which may be useful in studies of various metabolic systems (though not at present directly applicable to iron metabolism) are the limiting values for  $t$  very large, of the ratios  $\frac{q_2}{q_1}$  and  $\frac{q_2 Q_1}{q_1 Q_2}$ , i.e., of the ratios of the tracer amounts and of the specific activities, respectively, of the two pools. At the limit, when  $t$  becomes very large, the exponential function  $e^{-\lambda_1 t}$  becomes very small, and the ratio of the activities in the two pools approaches a constant  $L$ :

$$\frac{q_1}{q_2} \xrightarrow{t \rightarrow \infty} L = \sqrt{\frac{2A_{21}k_1}{k_1 + k_2}}.$$

Similarly, the ratio of the specific activities becomes

$$\frac{q_2 Q_1}{q_1 Q_2} \xrightarrow{t \rightarrow \infty} \sqrt{\frac{2A_{21}k_1}{k_1 + k_2}} \frac{Q_1}{Q_2}.$$

Note that this ratio differs with different models, and does not as a rule approach unity. In the special case of a closed system (Fig. 4), when  $E_1 = E_2 = S_1 = S_2 = 0$  and  $A_{21} = A_{12}A_{21} = A_{12} = 1$ , these equations reduce to

$$\frac{q_2}{q_1} \xrightarrow{t \rightarrow \infty} \frac{k_1}{k_2} = \frac{Q_1}{Q_2}$$

and

$$\frac{q_2 Q_1}{q_1 Q_2} \xrightarrow{t \rightarrow \infty} \frac{k_1 Q_1}{k_2 Q_2} = 1$$

respectively.

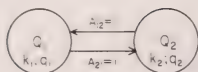


FIG. 4. Schematic representation of a closed two-pool system.

In the case of the above sixteen models, the value of  $\frac{Q_1}{Q_2}$  is obtained from Table IV, and  $A_{21}$  is calculated from the equation  $A_{21} = 1 - \epsilon_1(1 - A_{12}A_{21})$ . In general, however, when  $\epsilon_1$  is not given, the conditions of entry and exit can be expressed directly in terms of  $\sigma$ 's and  $\omega$ 's ( $1 \geq \sigma_i$ ,  $\omega_i \geq 0$  and  $\sum_i \sigma_i = \sum_i \omega_i = 1$ ). The constants  $A_{21}$  and  $\frac{A_{21}Q_1}{Q_2}$  can then be determined as functions of  $\sigma$ 's and  $\omega$ 's as follows: From the definitions, and the E.C. II

$$\sigma_1(A_{01}k_1Q_1 + A_{02}k_2Q_2) + A_{12}k_2Q_2 = k_1Q_1$$

and hence

$$\frac{Q_1k_1}{Q_2k_2} = \frac{A_{12}\sigma_2 + \sigma_1}{A_{21}\sigma_1 + \sigma_2}.$$

Similarly, from the definitions of  $\omega$ 's we get for  $\omega_1, \omega_2 \neq 0$  (and hence  $A_{01}, A_{02} \neq 0$ ):

$$\frac{\omega_1}{\omega_2} = \frac{A_{01}k_1Q_1}{A_{02}k_2Q_2}$$

or

$$\frac{Q_1k_1}{Q_2k_2} = \frac{\omega_1(1 - A_{12})}{\omega_2(1 - A_{21})}.$$

Equating the two expressions for  $\frac{Q_1 k_1}{Q_2 k_2}$  we finally obtain a quadratic equation for  $A_{21}$  in terms of  $\sigma_1$ ,  $\omega_1$ , and  $A_{12}A_{21}$  alone:

$$A_{21}\sigma_1 + A_{21}[\omega_1 - \sigma_1 + A_{12}A_{21}(1 - \sigma_1 - \omega_1)] - A_{12}A_{21}(1 - \sigma_1) = 0.$$

Note that this equation holds for all acceptable values of  $\sigma$ 's and  $\omega$ 's including zeros. Once  $A_{21}$  (and therefore  $A_{01}$ ,  $A_{02}$  and  $A_{12}$ ) has been expressed in the above terms, the physiological parameters  $R_e$  and  $Q_2$  of the most general two-pool model can be written in terms of  $\omega$ 's,  $\sigma$ 's, and the constants of the approximating equation:

From

$$\frac{Q_1 k_1}{Q_2 k_2} = \frac{A_{12}\sigma_2 + \sigma_1}{A_{21}\sigma_1 + \sigma_2}$$

it follows that

$$Q_2 = \frac{A_{21}\sigma_1 + \sigma_2}{A_{12}\sigma_2 + \sigma_1} \frac{k_1}{k_2} Q_1$$

and therefore

$$R_e = A_{01}k_1Q_1 + A_{02}k_2Q_2 = \left( A_{01} + \frac{A_{21}\sigma_1 + \sigma_2}{A_{12}\sigma_2 + \sigma_1} A_{02} \right) k_1Q_1.$$

In dealing with experimental data, it may be desirable so express the possible range of the limiting values of  $\frac{Q_2}{Q_1}$ , i.e., of  $L$ , as a function of  $k_1$  and  $k_2$  alone.

These limiting values may assume a special significance in distribution studies of such long life non-physiological tracers as radium or thorium. The explicit value of  $L$  was shown to be

$$L = \frac{2A_{21}k_1}{\sqrt{-(k_1 - k_2)}} = \frac{N}{D}.$$

For  $k_1 > k_2$  the maximum value  $2k_2$  of the denominator,  $D$ , is assumed when  $A_{12}A_{21} = 1$ . On the other hand  $D$  becomes arbitrarily small for sufficiently small  $A_{12}A_{21}$ , i.e., sufficiently small  $A_{12}$ . Therefore, given an appropriate model,  $L$  can assume any positive value such that

$$L > A_{21} \frac{k_1}{k_2}.$$

Similarly, when  $k_2 > k_1$ :

$$2(k_2 - k_1) < D \leq 2k_2$$

and

$$A_{21} \frac{k_1}{k_2} \leq L < \frac{A_{21}k_1}{k_2 - k_1} \leq \frac{k_1}{k_2 - k_1}.$$

Hence when  $k_2$  is very much larger than  $k_1$ ,  $L$  is approximately equal to  $A_{21} \frac{k_1}{k_2}$ :

$$L \approx A_{21} \frac{k_1}{k_2} \quad \text{for } k_2 \gg k_1.$$

Finally, when  $k_1 = k_2$ , the expression for  $L$  reduces to

$$L = \sqrt{\frac{A_{21}}{A_{12}}}.$$

In this latter case,  $L$  is independent of the rate constants and can assume any positive value for appropriate values of  $A_{21}$  and  $A_{12}$ .

Table IV demonstrates the fact that the value of  $\frac{Q_2}{Q_1}$  is a function of the particular model under consideration, and cannot be deduced directly from the approximating equation without further assumptions; yet a direct evaluation of  $\frac{Q_2}{Q_1}$  from the approximating curve is repeatedly attempted by workers in the field of tracer analysis. Such a direct evaluation of  $\frac{Q_2}{Q_1}$  is based on the assumption that the ratio of the sum of both pools to the first accessible pool is equal to the ratio of the 0-time intercept of the approximating curve to the 0-time intercept of the tangent to the final interval of such curve, i.e.,

$$\frac{Q_1 + Q_2}{Q_1} = \frac{\alpha_1 + \alpha_2}{\alpha_2} \quad \text{or} \quad \frac{Q_2}{Q_1} = \frac{\alpha_1}{\alpha_2} = \frac{a_1}{a_2},$$

where  $a_1$  and  $a_2$  are the coefficients of the normalized approximating equation

$$\frac{q_1}{q_0} = a_1 e^{-\lambda_1 t} + a_2 e^{-\lambda_2 t}.$$

The above equalities can be realized only under very special conditions. Let

$$F = \frac{Q_2}{Q_1} \frac{k_2}{k_1}$$

stand for the factors of Table IV, which describe the relationship between pools  $Q_2$  and  $Q_1$ , so that for each of the sixteen models the assumed equality reduces to

$$\frac{Q_2}{Q_1} = F \frac{k_1}{k_2} = \frac{a_1}{a_2} = \frac{\sqrt{} + (k_1 - k_2)}{\sqrt{} - (k_1 - k_2)}$$

or

$$(Fk_1 - k_2) \sqrt{} = (k_1 - k_2)(Fk_1 + k_2).$$

(Note that this equality is satisfied in the special case of a closed system, i.e., when  $F = 1$  and  $\sqrt{} = k_1 + k_2$ ). In general, when  $k_1 = k_2$ , the equality is sat-



ified for  $F = 1$ , i.e., only for models Ib, IIa, IIId and IVc of Table III. When  $k_1 \neq k_2$ , the expression  $(Fk_1 - k_2)$  in the last equality cannot be zero, and  $\sqrt{\quad}$  can be written as

$$\sqrt{\quad} = k_1 - k_2 + \frac{2k_2(k_1 - k_2)}{Fk_1 - k_2} = k_2 - k_1 + \frac{2k_1F(k_2 - k_1)}{k_2 - Fk_1}.$$

Since in this case  $\sqrt{\quad} < k_1 + k_2$ , it follows that

$$\frac{k_1 - k_2}{Fk_1 - k_2} < 1 \quad \text{and} \quad \frac{k_2 - k_1}{\frac{k_2}{F} - k_1} < 1.$$

Therefore, when  $k_1 > k_2$ ,  $F$  must satisfy the condition  $Fk_1 - k_2 > k_1 - k_2$ , i.e.,  $F > 1$ , which automatically eliminates from consideration all models of Table III except IIb, c, d, IIb, c, and IVb, d. Similarly, when  $k_1 < k_2$ , it follows that  $F < 1$ , which in turn eliminates all models except Ia, c, d, IIIa, c, and IVa, d. It should be noted that even when a solution is possible for a given model and particular values of  $k_1$  and  $k_2$ , the equality

$$\frac{a_1}{a_2} = F \frac{k_1}{k_2}$$

can be satisfied only for special values of the interchange constant  $A_{12}A_{21}$ . Hence  $\frac{Q_2}{Q_1}$  cannot in general be derived from the constants of the approximating curve.

In the preceding, the two arbitrary conditions necessary to determine the two-pool system completely, were expressed in terms of the modes of entry and exit, i.e., in terms of  $\sigma$ 's and  $\omega$ 's. Such an approach is especially well adapted to studies of iron metabolism where the data on maximum uptake,  $U$ , are readily available. It should be borne in mind, however, that the above procedure is general, and that though a large variety of pairs of two independent conditions may be used, each such pair can be reduced to conditions expressed in terms of  $\sigma$ 's and  $\omega$ 's. For instance, if the first condition is one of the  $A$ 's ( $A_{12}A_{21}$  and either  $A_{01}$ ,  $A_{21}$ ,  $A_{02}$  or  $A_{12}$  define all remaining  $A$ 's), the second can be one of the following:  $Q_2$ ,  $E_1$ ,  $E_1 + E_2$ ,  $E_2$ ,  $S_1$ ,  $S_1 + S_2$ ,  $S_2$ ,  $\sigma_1$ ,  $\sigma_2$ ,  $\omega_1$  or  $\omega_2$ . If the second condition is  $Q_2$ , it follows from E.C. II that  $S_1 = k_1Q_1 - A_{12}k_2Q_2$ . On the other hand  $E_1 = A_{01}k_1Q_1$  and  $E_2 = A_{02}k_2Q_2$ . From  $S_1$ ,  $E_1$  and  $E_2$ , the values of  $\sigma$ 's and  $\omega$ 's follow directly.

Similarly, the two conditions can be given by  $Q_2$  and one of the following:  $E_1$ ,  $E_1 + E_2$ ,  $E_2$ ,  $S_1$ ,  $S_1 + S_2$ ,  $S_2$ ,  $\sigma_1$ ,  $\sigma_2$ ,  $\omega_1$ ,  $\omega_2$ , or by such combinations as  $E_1$  and  $S_1$ , etc.

#### *Section 4. Plasma iron comprises two spatially indistinguishable interchanging iron pools.*

Until now it was assumed that the two iron pools  $Q_1$  and  $Q_2$  were spatially separated. We will now consider the case where both pools are contained in the

plasma iron, and where, therefore, individual sampling of either of the two pools is no longer possible. This problem can be easily solved if the relative amounts of the tracer introduced initially into each of the two pools is known.

Let fraction  $\gamma_1$  of the initial tracer dose  $q_0$  go into the first pool  $Q_1$ , and fraction  $\gamma_2 = 1 - \gamma_1$ , into  $Q_2$  at the time  $t = 0$ . The above numbering of the pools is arbitrary. Making use of the superposition principle and summing up the contributions of initial tracer activities to  $q_1$  and  $q_2$  respectively, we get:

$$(1) \quad \frac{q_1}{q_0} = \frac{\gamma_1[\sqrt{\quad} + (k_1 - k_2)] - 2\gamma_2 A_{12} k_2}{2\sqrt{\quad}} e^{-\lambda_1 t} + \frac{\gamma_1[\sqrt{\quad} - (k_1 - k_2)] + 2\gamma_2 A_{12} k_2}{2\sqrt{\quad}} e^{-\lambda_2 t}$$

$$(2) \quad \frac{q_2}{q_0} = \frac{\gamma_2[\sqrt{\quad} + (k_2 - k_1)] - 2\gamma_1 A_{21} k_1}{2\sqrt{\quad}} e^{-\lambda_1 t} + \frac{\gamma_2[\sqrt{\quad} - (k_2 - k_1)] - 2\gamma_1 A_{21} k_1}{2\sqrt{\quad}} e^{-\lambda_2 t}$$

$$(3) \quad \frac{q_T}{q_0} = \frac{q_1 + q_2}{q_0} = a'_1 e^{-\lambda_1 t} + a'_2 e^{-\lambda_2 t}$$

where  $\sqrt{\quad}$ ,  $\lambda_1$  and  $\lambda_2$  are defined as before and

$$(4) \quad a'_1 = \frac{\sqrt{\quad} + \gamma_1(k_1 - 2A_{21}k_1 - k_2) + \gamma_2(k_2 - 2A_{12}k_2 - k_1)}{2\sqrt{\quad}}$$

$$(5) \quad a'_2 = \frac{\sqrt{\quad} + \gamma_1(k_2 + 2A_{21}k_1 - k_1) + \gamma_2(k_1 + 2A_{12}k_2 - k_2)}{2\sqrt{\quad}}$$

In general, although  $a'_1 + a'_2 = 1$ ,  $a'_1$  can assume negative values for certain admissible values of the pool constants. Since in practice the plasma iron disappearance curves are concave with no inflection points when plotted on semilog paper (i.e., when the dependent variable is the logarithm of  $q_T$ ), we will restrict all subsequent considerations primarily to cases for which both  $a'_1$  and  $a'_2$  are positive. We will further assume that  $0 < 1 - A_{12}A_{21} \leq 1$ , since  $1 - A_{12}A_{21} = 0$  implies a closed system with a constant value of  $q_T = q_1 + q_2$ . Note that  $1 - A_{12}A_{21} = 1$  indicates either unidirectional flow or the existence of two independent systems, which will be considered in the next section. The values of  $k_1$ ,  $k_2$  and  $A_{12}A_{21}$  cannot be derived from constants  $a'_1$ ,  $a'_2$ ,  $\lambda_1$  and  $\lambda_2$  of the approximating equation without some further assumptions about  $A_{12}$  and  $A_{21}$ , or their equivalents. Before imposing any restrictions upon the system, it will be convenient to introduce a few relations which will prove useful subsequently:

$$(6) \quad \lambda_1 + \lambda_2 = k_1 + k_2 \quad \text{or} \quad k_1 = \lambda_1 + \lambda_2 - k_2$$

$$(7) \quad \lambda_1 - \lambda_2 = \sqrt{\quad} \neq 0$$

(As previously noted, in a two-pool system  $\lambda_1 = \lambda_2$  only in the case of unidirectional flow with  $k_1 = k_2$ .)

$$(8) \quad \lambda_1 \lambda_2 = (1 - A_{12} A_{21}) k_1 k_2$$

or

$$(1 - A_{12} A_{21}) = \frac{\lambda_1 \lambda_2}{k_1 k_2},$$

since  $1 - A_{12} A_{21} \neq 0$ .

$$(9) \quad a'_1 + a'_2 = 1$$

$$(10) \quad a'_1 - a'_2 = \frac{\gamma_1(k_1 - 2A_{21}k_1 - k_2) + \gamma_2(k_2 - 2A_{12}k_2 - k_1)}{\sqrt{\quad}}$$

$$(11) \quad (a'_1 - a'_2)(\lambda_1 - \lambda_2) = \gamma_1(k_1 - 2A_{21}k_1 - k_2) + \gamma_2(k_2 - 2A_{12}k_2 - k_1).$$

These formulae can now be applied to models of Section 3. Since the numbering of the two pools is arbitrary, it suffices to consider only Groups I and III.

*Group I:*

In this group,  $e_1 = 1$ ,  $A_{12} = 1$  and  $A_{21} = A_{12}A_{21}$ . Equation (11) therefore becomes

$$(11a) \quad (a'_1 - a'_2)(\lambda_1 - \lambda_2) = 2\gamma_1 k_1 (1 - A_{12} A_{21}) - (k_1 + k_2).$$

From this and equations (6) and (8) it follows that

$$(12a) \quad (a'_1 - a'_2)(\lambda_1 - \lambda_2) + \lambda_1 + \lambda_2 = 2\gamma_1 k_1 (1 - A_{12} A_{21}) = \frac{2\gamma_1 \lambda_1 \lambda_2}{k_2},$$

$$(13a) \quad k_2 = \frac{2\gamma_1 \lambda_1 \lambda_2}{(a'_1 - a'_2)(\lambda_1 - \lambda_2) + \lambda_1 + \lambda_2} = \frac{\gamma_1 \lambda_1 \lambda_2}{a'_1 \lambda_1 + a'_2 \lambda_2}$$

$$\gamma_1 = \frac{k_2(a'_1 \lambda_1 + a'_2 \lambda_2)}{\lambda_1 \lambda_2}.$$

Since by definition

$$0 < (1 - A_{12} A_{21}) = \frac{\lambda_1 \lambda_2}{k_1 k_2} \leq 1$$

and  $\lambda_1 > \lambda_2$ , and since  $k_1 + k_2 = \lambda_1 + \lambda_2$ , the following inequalities must be satisfied by the constants of the models belonging to Group I:

$$(14a) \quad \lambda_2 \leq k_1 \leq \lambda_1 \quad \text{and} \quad \lambda_2 \leq k_2 \leq \lambda_1,$$

and therefore

$$\lambda_2 \leq \frac{\gamma_1 \lambda_1 \lambda_2}{a'_1 \lambda_1 + a'_2 \lambda_2} \leq \lambda_1.$$

Hence, since all constants were assumed positive,

$$\frac{a'_1\lambda_1\lambda_2 + a'_2\lambda_2^2}{\lambda_1\lambda_2} \leq \gamma_1 \leq \frac{a'_1\lambda_1^2 + a'_2\lambda_1\lambda_2}{\lambda_1\lambda_2}$$

or

$$(15a) \quad a'_1 + a'_2 \frac{\lambda_2}{\lambda_1} \leq \gamma_1 \leq a'_2 + a'_1 \frac{\lambda_1}{\lambda_2};$$

and since by definition

$$\gamma_1 \leq 1 \quad \text{and} \quad a'_2 + a'_1 \frac{\lambda_1}{\lambda_2} > 1,$$

it follows that

$$(16a) \quad a'_1 + a'_2 \frac{\lambda_2}{\lambda_1} \leq \lambda_1 \leq 1.$$

For a given  $\gamma_1$ , provided it satisfies the inequality (16a), the constants  $k_1$ ,  $k_2$  and  $A_{12}A_{21} = A_{21}$  can now be determined from equations (13a), (6) and (8). These constants are the same for all four models of Group I. It remains to determine

$$(17a) \quad Q_1 = Q_T \frac{Q_1}{Q_1 + Q_2}$$

as a function of the total plasma iron  $Q_T = Q_1 + Q_2$  and the pool constants  $k_1$ ,  $k_2$  and  $A_{12}A_{21}$ . The constant  $Q_2$  is then simply  $Q_2 = Q_T - Q_1$ , while the remaining clinical constants can be obtained from Table IV.

Case 1:  $\gamma_1 = 1$ .

If  $\gamma_1 = 1$ , the inequality (16a) is always satisfied and  $Q_1$  is obtained by substituting into the identity (17a) the  $Q_2$  value of the corresponding model of Table IV.

Model Ia:

$$Q_1 = Q_T \left( \frac{Q_1}{Q_1 + \frac{k_1}{k_2} A_{12}A_{21}Q_1} \right) = \frac{Q_T}{1 + \frac{k_1}{k_2} A_{12}A_{21}}.$$

Model Ib:

$$Q_1 = \frac{Q_T}{1 + \frac{k_1}{k_2}}.$$

Model Ic:

$$Q_1 = \frac{Q_T}{1 + [1 - U(1 - A_{12}A_{21})] \frac{k_1}{k_2}}.$$

Model Id:

$$Q_1 = \frac{Q_T}{1 + [A_{12}A_{21} + U(1 - A_{12}A_{21})] \frac{k_1}{k_2}}.$$

Case 2:  $\gamma_1 = 0$ .

The inequality (16a) cannot be satisfied by  $\gamma_1 = 0$  and therefore no solutions exist for positive  $a'_1$ . However, if  $a'_1$  is negative and inequality (15a) is satisfied, solutions can be found by methods similar to those of Case I using the identity (17a).

$$\text{Case 3: } \gamma_1 = \frac{Q_1}{Q_1 + Q_2}.$$

This is equivalent to the assumption that  $\gamma_1:\gamma_2 = Q_1:Q_2$ , which leads to different  $\gamma$ 's and hence different sets of constants for each model, provided the inequality (16a) is satisfied.  $\gamma_1$  can then be written as

$$\gamma_1 = \frac{k_2(a'_1\lambda_1 + a'_2\lambda_2)}{\lambda_1\lambda_2} = \frac{Q_1}{Q_1 + Q_2} \quad (\text{from equation 13a}).$$

Model Ia:

$$\gamma_1 = \frac{Q_1}{Q_1 + Q_2} = \frac{1}{1 + \frac{k_1}{k_2} A_{12}A_{21}} = \frac{k_2(a'_1\lambda_1 + a'_2\lambda_2)}{\lambda_1\lambda_2};$$

this reduces to

$$k_2 = \frac{\lambda_1\lambda_2}{\lambda_1 + \lambda_2 - \frac{\lambda_1\lambda_2}{a'_1\lambda_1 + a'_2\lambda_2}}.$$

If the inequality (16a) is satisfied, the value of  $k_2$  lies between  $\lambda_1$  and  $\lambda_2$ ;  $k_1$  and  $A_{12}A_{21}$  follow from equations (6) and (8), and  $Q_1$  can then be determined as above.

Model Ib:

$$\gamma_1 = \frac{1}{1 + \frac{k_1}{k_2}} = \frac{k_2}{\lambda_1 + \lambda_2} = \frac{k_2(a'_1\lambda_1 + a'_2\lambda_2)}{\lambda_1\lambda_2}.$$

Hence

$$\lambda_1\lambda_2 = (\lambda_1 + \lambda_2)(a'_1\lambda_1 + a'_2\lambda_2) = a'_1\lambda_1^2 + \lambda_1\lambda_2 + a'_2\lambda_2^2.$$

This is impossible for positive values of  $a'_1$  and  $a'_2$ . If  $a'_1$  is negative, a solution can be found for any value of  $k_2$  such that  $\lambda_1 > k_2 > \lambda_2$  and for which

$$\gamma_1 = \frac{k_2}{\lambda_1 + \lambda_2}$$

satisfies the inequality (15a).

Model Ic:

$$\gamma_1 = \frac{1}{1 + [1 - U(1 - A_{12}A_{21})] \frac{k_1}{k_2}} = \frac{k_2(a'_1\lambda_1 + a'_2\lambda_2)}{\lambda_1\lambda_2}$$

and

$$k_2 = \frac{U\lambda_1\lambda_2}{\lambda_1 + \lambda_2 - \frac{\lambda_1\lambda_2}{a'_1\lambda_1 + a'_2\lambda_2}}.$$

Model Id:

$$\gamma_1 = \frac{1}{1 + [A_{12}A_{21} + U(1 - A_{12}A_{21})] \frac{k_1}{k_2}} = \frac{k_2(a'_1\lambda_1 + a'_2\lambda_2)}{\lambda_1\lambda_2}$$

and

$$k_2 = \frac{(1 - U)\lambda_1\lambda_2}{\lambda_1 + \lambda_2 - \frac{\lambda_1\lambda_2}{a'_1\lambda_1 + a'_2\lambda_2}}.$$

### Group III

For this group  $U = e_1$ , and therefore

$$A_{21} = 1 - U(1 - A_{12}A_{21}) = 1 - U \frac{\lambda_1\lambda_2}{k_1k_2}$$

$$A_{12} = \frac{A_{12}A_{21}}{1 - U(1 - A_{12}A_{21})} = \frac{k_1k_2 - \lambda_1\lambda_2}{k_1k_2 - U\lambda_1\lambda_2}.$$

Substituting these values for  $A_{21}$  and  $A_{12}$  in equation (11) and simplifying, gives

$$(12b) \quad \frac{a'_1\lambda_1 + a'_2\lambda_2}{\lambda_1\lambda_2} = \frac{U\gamma_1}{k_2} + \frac{(1 - U)(1 - \gamma_1)k_2}{k_2(\lambda_1 + \lambda_2 - k_2) - U\lambda_1\lambda_2}.$$

When  $U = 1$  this reduces to equation (13a) and only models Ia and Ib need be considered.

Case 1:  $\gamma_1 = 1$

When  $\gamma_1 = 1$  equation (12b) becomes

$$(13b) \quad k_2 = \frac{U\lambda_1\lambda_2}{a'_1\lambda_1 + a'_2\lambda_2}$$

with the corresponding inequality

$$(16b) \quad a'_1 + a'_2 \frac{\lambda_2}{\lambda_1} \leq U \leq 1 < a'_2 + a'_1 \frac{\lambda_1}{\lambda_2}$$

for



$$a'_1 > 0; \quad \lambda_1 \neq \lambda_2.$$

When  $U$  satisfies the above inequality, the values of  $k_1$ ,  $k_2$  and  $A_{12}A_{21}$  can be obtained from equations (13b), (6) and (8), and that of  $Q_1$  can then be determined individually for each model as described in Case 1, Group I.

Case 2:  $\gamma_1 = 0$

When  $\gamma_1 = 0$

$$(13c) \quad k_2^2 - \frac{a'_1\lambda_1^2 + a'_2\lambda_2^2 - U\lambda_1\lambda_2}{a'_1\lambda_1 + a'_2\lambda_2} k_2 + U\lambda_1\lambda_2 = 0.$$

The corresponding inequalities limiting the possible values of  $U$  involve a function of  $A_{12}A_{21}$  and  $U$  (or  $k_2$  and  $U$ ):

$$(15c) \quad a'_1 + a'_2 \frac{\lambda_2}{\lambda_1} \leq \frac{1 - U}{\frac{1}{1 - A_{12}A_{21}} - U} \leq a'_2 + a'_1 \frac{\lambda_1}{\lambda_2}$$

$$(16c) \quad a'_1 + a'_2 \frac{\lambda_2}{\lambda_1} \leq \frac{1 - U}{\frac{1}{1 - A_{12}A_{21}} - U} \leq 1 \quad \text{for } a'_1 > 0.$$

It may, therefore, be simpler to state that solutions can be found for any value of  $U$  for which  $k_2^2$  satisfies the inequality

$$(14a) \quad \lambda_2 \leq k_2 \leq \lambda_1$$

$$\text{Case 3: } \gamma_1 = \frac{Q_1}{Q_1 + Q_2}.$$

Here again the procedure is similar to that for Group I. For instance, consider Model IIIa. Then

$$\gamma_1 = \frac{1}{1 + [1 - U(1 - A_{12}A_{21})] \frac{k_1}{k_2}}$$

Using equations (6) and (8) this reduces to

$$\gamma_1 = \frac{k_2}{(k_1 + k_2) - k_1 U(1 - A_{12}A_{21})} = \frac{k_2^2}{k_2(\lambda_1 + \lambda_2) - U\lambda_1\lambda_2}.$$

Substituting this value of  $\gamma_1$  into (12b) we can solve for  $k_2$  in terms of any given  $\lambda_1$ ,  $\lambda_2$ ,  $a'_1$  and  $a'_2$ . If  $k_2$  satisfies the inequality (14a),  $k_1$ ,  $A_{12}A_{21}$ , and  $Q_1$  can then be determined as before.

*Section 5. Plasma iron consists of two independent spatially indistinguishable single-pool systems.*

The solution for  $R_e$  is indeterminate unless amounts of stable iron in the two pools can be determined separately. From the solution for  $q_T = q_1 + q_2$  it is pos-

sible to derive only the individual relative rates  $k_1$  and  $k_2$ , and the fractions  $\gamma_1$  and  $\gamma_2$  of the tracer  $q_0$  initially introduced into the pools  $Q_1$  and  $Q_2$  respectively.

$$\frac{q_T}{q_0} = \frac{q_1 + q_2}{q_0} = \gamma_1 e^{-k_1 t} + \gamma_2 e^{-k_2 t}.$$

If it is assumed that  $\gamma_1 : \gamma_2 = Q_1 : Q_2$ , then  $Q_1 = Q_T \gamma_1$  and  $Q_2 = Q_T \gamma_2$ , where  $Q_T$  stands for the total plasma iron. The total amount of iron leaving plasma per unit time can then be expressed as

$$R_i = k_1 Q_1 + k_2 Q_2 = Q_T (\gamma_1 k_1 + \gamma_2 k_2).$$

### Section 6. Accumulation pools.

In all previous discussions a positive constant  $k_i$  has been associated with each pool  $Q_i$  of a given system. This condition excluded from the above considerations what may be called "accumulation" pools, i.e., excretion and permanent storage or deposit pools, for which  $k_i = 0$ . The reason for this omission was twofold. Firstly, such pools are not subject to the equilibrium conditions as defined above. Secondly, the inclusion or exclusion of such pools in no way alters the number or character of the exponential components of the tracer equations for the remaining pools. In some cases, however, analysis of an "accumulation" pool may yield as much, or even more, information about the system as may be obtained from sampling one of its pools  $Q_i$ . In case of a single-pool system, the solution of the problem is immediate. The total cumulative amount of tracer excreted up to a given time  $t$ ,  $e(t)$ , is given by

$$e(t) = q_0 k \int_0^t e^{-kt} dt = q_0 (1 - e^{-kt});$$

the remaining activity,  $q_0$  minus the experimental (cumulative values, can then be approximated by a single exponential function  $q = q_0 e^{-kt}$ . If the total amount  $E$  of the substance excreted per unit time is known,  $Q$  follows from  $E = kQ$  and the system is completely determined.

In the case of a two-pool system, three cases of "accumulation" pools can be differentiated:

1. The "accumulation" pool eventually contains all tracer which leaves the system through the first pool. Then:

$$e_1(t) = k_1 A_{01} q_0 \int_0^t (a_1 e^{-\lambda_1 t} + a_2 e^{-\lambda_2 t}) dt$$

$$e_1(t) = \delta' [1 - \alpha'_1 e^{-\lambda_1 t} - \alpha'_2 e^{-\lambda_2 t}]$$

where  $\lambda_1, \lambda_2, a_1$  and  $a_2$  are defined as before, and

$$\delta' = \frac{k_1 A_{01} q_0 (a_1 \lambda_2 + a_2 \lambda_1)}{\lambda_1 \lambda_2} = \frac{A_{01} q_0}{1 - A_{12} A_{21}} = e_1(t)_{t \rightarrow \infty}$$

$$\alpha'_1 = \frac{a_1 \lambda_2}{a_1 \lambda_2 + a_2 \lambda_1} = \frac{a_1 k_1 (1 - A_{12} A_{21})}{\lambda_1}$$

$$\alpha'_2 = \frac{a_2 \lambda_1}{a_1 \lambda_2 + a_2 \lambda_1} = \frac{a_2 k_1 (1 - A_{12} A_{21})}{\lambda_2}$$

The approximating equation yields four (instead of the usual three) independent constants  $\lambda_1$ ,  $\lambda_2$ ,  $\alpha'_1$  and  $\delta'$  with the following relations to the pool constants:

$$\lambda_1 + \lambda_2 = k_1 + k_2$$

$$\lambda_1 \lambda_2 = k_1 k_2 (1 - A_{12} A_{21})$$

$$\lambda_1 - \lambda_2 = \sqrt{(k_1 + k_2)^2 - 4(1 - A_{12} A_{21}) k_1 k_2} = \sqrt{\quad}$$

In addition

$$\alpha'_1 + \alpha'_2 = 1$$

and

$$\alpha'_1 - \alpha'_2 = \frac{2\lambda_1 \lambda_2 - k_2(\lambda_1 + \lambda_2)}{(\lambda_1 - \lambda_2)k_2}.$$

Therefore,

$$k_2(\lambda_1 - \lambda_2)(\alpha'_1 - \alpha'_2) + k_2(\lambda_1 + \lambda_2) = 2\lambda_1 \lambda_2$$

and

$$k_2 = \frac{2\lambda_1 \lambda_2}{(\lambda_1 - \lambda_2)(\alpha'_1 - \alpha'_2) + \lambda_1 + \lambda_2} = \frac{\lambda_1 \lambda_2}{\alpha_1 \lambda_1 + \alpha_2 \lambda_2}.$$

$k_1$  and  $A_{12} A_{21}$  can now be calculated as before. In addition, the values of  $A_{01}$ ,  $A_{21}$ ,  $A_{02}$  and  $A_{12}$  follow from  $\delta'$  and  $(1 - A_{12} A_{21})$ . For models of Group I,  $\delta' = q_0$ . If  $E$  is known, the value of  $Q_1$  follows from  $E_1 = A_{01} k_1 Q_1$ . The two-pool system now possesses only one degree of freedom which can be expressed as the mode of entry.

2. The "accumulation" pool eventually contains all tracer which leaves the system through the second pool:

$$e_2(t) = k_2 A_{02} \frac{k_1 A_{21} q_0}{\sqrt{\quad}} \int_0^t (e^{-\lambda_2 t} - e^{-\lambda_1 t}) dt = \delta'' (1 + \alpha_1'' e^{-\lambda_1 t} - \alpha_2'' e^{-\lambda_2 t})$$

where

$$\delta'' = \frac{A_{21} A_{02}}{1 - A_{12} A_{21}} q_0$$

$$\alpha_1'' = \frac{\lambda_2}{\lambda_1 - \lambda_2}$$

$$\alpha_2'' = \frac{\lambda_1}{\lambda_1 - \lambda_2}$$

and

$$\alpha_2'' - \alpha_1'' = 1.$$

Here the approximating equation yields only three independent constants  $\lambda_1$ ,  $\lambda_2$  and

$$\frac{A_{21}A_{02}}{1 - A_{12}A_{21}},$$

and therefore the system possesses two degrees of freedom.

3. The "accumulation" pool ultimately contains all tracer which leaves the two-pool system:

$$e_T(t) = e_1(t) + e_2(t) = q_0(1 - \alpha_1''' e^{-\lambda_1 t} - \alpha_2''' e^{-\lambda_2 t})$$

where

$$\begin{aligned}\alpha_1''' &= \frac{A_{01}a_1k_1}{\lambda_1} - \frac{A_{21}A_{02}}{(1 - A_{12}A_{21})} \frac{\lambda_2}{(\lambda_1 - \lambda_2)} \\ \alpha_2''' &= \frac{A_{01}a_2k_1}{\lambda_2} + \frac{A_{21}A_{02}}{(1 - A_{12}A_{21})} \frac{\lambda_1}{(\lambda_1 - \lambda_2)} \\ \alpha_1''' + \alpha_2''' &= \frac{A_{01}}{1 - A_{12}A_{21}} + \frac{A_{21}A_{02}}{1 - A_{12}A_{21}} = 1.\end{aligned}$$

It follows that

$$\alpha_1''' - \alpha_2''' = \frac{2A_{01}k_1 - (\lambda_1 + \lambda_2)}{\lambda_1 - \lambda_2},$$

and therefore,

$$A_{01}k_1 = \frac{(\lambda_1 - \lambda_2)(a_1''' - a_2''') + \lambda_1 + \lambda_2}{2} = \lambda_1\alpha_1''' + \lambda_2\alpha_2'''.$$

Here again, two degrees of freedom remain.

This concludes the discussion of the general concepts and methods of multiple-pool analysis with special reference to various two-pool models. More complex systems are presented in "Multiple-Pool Analysis in Tracer Studies of Metabolic Kinetics" Part II: "Three-Pool Models and Partial Systems"<sup>(76)</sup>.

#### APPENDIX

The solution of the two simultaneous first order linear homogeneous differential equations with constant coefficients (corresponding to a two-pool system (Fig. 3))

$$(1) \quad \frac{dq_1}{dt} = -k_1q_1 + A_{12}k_2q_2$$

$$(2) \quad \frac{dq_2}{dt} = A_{21}k_1q_1 - k_2q_2$$

can be obtained by following the outline in any elementary text on ordinary differential equations. Differentiating the second equation with respect to  $t$  and

eliminating terms containing  $q_1$  we get the second order differential equation

$$(3) \quad \frac{d^2 q_2}{dt^2} + (k_1 + k_2) \frac{dq_2}{dt} + (1 - A_{12}A_{21})k_1k_2q_2 = 0$$

The corresponding auxiliary equation

$$(4) \quad m^2 + (k_1 + k_2)m + (1 - A_{12}A_{21})k_1k_2 = 0$$

has the two roots

$$(5) \quad r_1 = \frac{-(k_1 + k_2) - \sqrt{(k_1 + k_2)^2 - 4(1 - A_{12}A_{21})k_1k_2}}{2} = -\lambda_1$$

and

$$(6) \quad r_2 = \frac{-(k_1 + k_2) + \sqrt{(k_1 + k_2)^2 - 4(1 - A_{12}A_{21})k_1k_2}}{2} = -\lambda_2.$$

Case 1. When  $r_1$  and  $r_2$ , i.e.,  $-\lambda_1$  and  $-\lambda_2$  are distinct ( $\sqrt{\quad} \neq 0$  when either  $k_1 \neq k_2$  or  $A_{12}A_{21} \neq 0$  or both) the general solution for  $q_2$  becomes

$$(7) \quad q_2(t) = C_1 e^{r_1 t} + C_2 e^{r_2 t} = C_1 e^{-\lambda_1 t} + C_2 e^{-\lambda_2 t},$$

where  $C_1$  and  $C_2$  are arbitrary constants.

From equation (2) it follows that

$$q_1 = \frac{\frac{dq_2}{dt} + k_2 q_2}{A_{21}k_1}.$$

Hence substituting the solution for  $q_2$  in the above, we get the general solution for  $q_1$ :

$$(8) \quad q_1(t) = \frac{1}{A_{21}k_1} [C_1(k_2 - \lambda_1)e^{-\lambda_1 t} + C_2(k_2 - \lambda_2)e^{-\lambda_2 t}] \quad \text{for } A_{21}k_1 \neq 0.$$

The initial conditions are given as  $q_1(0) = q_0$  and  $q_2(0) = 0$ . Hence when  $t = 0$  equations (7) and (8) become

$$0 = C_1 + C_2$$

$$q_0 = \frac{1}{A_{21}k_1} [(k_2 - \lambda_1)C_1 + (k_2 - \lambda_2)C_2]$$

and therefore,

$$C_1 = -\frac{q_0 A_{21}k_1}{\lambda_1 - \lambda_2} = -q_0 \frac{A_{21}k_1}{\sqrt{\quad}}$$

$$C_2 = q_0 \frac{A_{21}k_1}{\sqrt{\quad}}.$$

The final tracer equations can then be written as

$$(9) \quad \frac{q_1(t)}{q_0} = \frac{\sqrt{-}}{2\sqrt{-}} + \frac{(k_1 - k_2)}{2\sqrt{-}} e^{-\lambda_1 t} + \frac{\sqrt{-}}{2\sqrt{-}} - \frac{(k_1 - k_2)}{2\sqrt{-}} e^{-\lambda_2 t}$$

$$(10) \quad \frac{q_2(t)}{q_0} = \frac{A_{21}k_1}{\sqrt{-}} (e^{-\lambda_2 t} - e^{-\lambda_1 t}).$$

When the flow is unidirectional (Fig. 5), i.e., when  $A_{12} = 0$  (and therefore  $A_{12}A_{21} = 0$ ), the roots of the auxiliary equation (4) become

$$(5a) \quad r_1 = -k_1 = -\lambda_1$$

$$(6a) \quad r_1 = -k_2 = -\lambda_2,$$

and the corresponding solutions reduce to

$$(9a) \quad \frac{q_1(t)}{q_0} = e^{-k_1 t}$$

$$(10a) \quad \frac{q_2(t)}{q_0} = \frac{A_{21}k_1}{k_1 - k_2} (e^{-k_2 t} - e^{-k_1 t}).$$

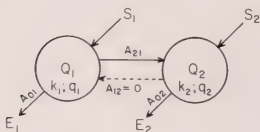


FIG. 5. Schematic representation of a unidirectionally connected two-pool system.

Case 2. When in addition to  $A_{12} = 0$  (unidirectional flow),  $k_1 = k_2 = k$ , the discriminant of the auxiliary equation (4) becomes zero ( $\sqrt{-} = 0$ ) and

$$r_1 = r_2 = r \quad \text{or} \quad -\lambda_1 = -\lambda_2 = -k.$$

In this case the general solutions become:

$$(8b) \quad q_1(t) = \frac{B_2 e^{-kt}}{A_{21}k}$$

$$(7b) \quad q_2(t) = B_1 e^{-kt} + B_2 t e^{-kt}.$$

Substituting initial conditions we get

$$0 = B_1$$

$$q_0 = \frac{B_2}{A_{21}k},$$

and therefore

$$B_1 = 0$$

$$B_2 = q_0 A_{21}k.$$



Hence the solutions become:

$$(9b) \quad \frac{q_1(t)}{q_0} = e^{-kt}$$

$$(10b) \quad \frac{q_2(t)}{q_0} = A_{21}kte^{-kt}.$$

#### REFERENCES

A selected bibliography of source material is presented at the end of "Multiple-Pool Analysis in Tracer Studies of Metabolic Kinetics: II." A limited number of direct references will be made where specifically indicated.

# Multiple-Pool Analysis in Tracer Studies of Metabolic Kinetics: II

## Three-pool Models and Partial Systems\*

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The fundamental concepts and definitions were presented in "Multiple-Pool Analysis in Tracer Studies of Metabolic Kinetics: I. (Two-Pool Models)"<sup>75</sup>. More complex systems are considered below.

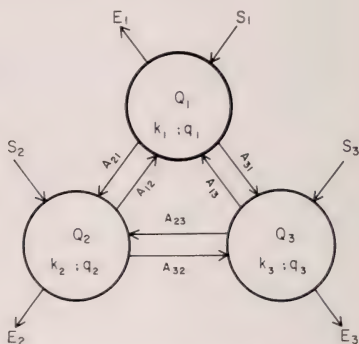
### THREE-POOL SYSTEMS

The analysis of a reduced three-pool system (i.e., a three-pool system where some of the substance moves from any given pool to another either directly or by means of an intermediary pool) may proceed according to the general plan previously outlined<sup>75</sup>. Let  $Q_1$ ,  $Q_2$  and  $Q_3$  be the three pools of a reduced system and let  $Q_1$  be the accessible pool (Fig. 1).

Then:

$$\frac{dq_1}{dt} = -k_1q_1 + A_{12}k_2q_2 + A_{13}k_3q_3$$

FIG. 1. Schematic representation of a general three-pool system.



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$$\frac{dq_2}{dt} = A_{21}k_1q_1 - k_2q_2 + A_{23}k_3q_3$$

$$\frac{dq_3}{dt} = A_{31}k_1q_1 + A_{32}k_2q_2 - k_3q_3.$$

$$D(p) = \begin{vmatrix} p + k_1 & -A_{12}k_2 & -A_{13}k_3 \\ -A_{21}k_1 & p + k_2 & -A_{23}k_3 \\ -A_{31}k_1 & -A_{32}k_2 & p + k_3 \end{vmatrix} = (p + \lambda_1)(p + \lambda_2)(p + \lambda_3) = 0$$

$$= p^3 + p^2(k_1 + k_2 + k_3) + p[k_1k_2(1 - A_{12}A_{21}) + k_2k_3(1 - A_{23}A_{32}) + k_1k_3(1 - A_{13}A_{31})] + k_1k_2k_3(1 - A_{12}A_{21} - A_{23}A_{32} - A_{13}A_{31} - A_{12}A_{23}A_{31} - A_{13}A_{32}A_{21}) = p^3 + s_1p^2 + s_2p + s_3.$$

$$D'(p) = 3p^2 + 2s_1p + s_2$$

$$= (p + \lambda_2)(p + \lambda_3) + (p + \lambda_1)(p + \lambda_3) + (p + \lambda_1)(p + \lambda_2).$$

$$N_1(p) = \begin{vmatrix} q_0 & -A_{12}k_2 & -A_{13}k_3 \\ 0 & p + k_2 & -A_{23}k_3 \\ 0 & -A_{32}k_2 & p + k_3 \end{vmatrix} = q_0 \begin{vmatrix} p + k_2 & -A_{23}k_3 \\ -A_{32}k_2 & p + k_3 \end{vmatrix}$$

$$= q_0[(p + k_2)(p + k_3) - A_{23}A_{32}k_2k_3]$$

$$= q_0[p^2 + p(k_2 + k_3) + k_2k_3(1 - A_{23}A_{32})]$$

The general solution for  $\frac{q_1}{q_0}$  can then be written directly:

$$\frac{q_1}{q_0} = \sum_{i=1}^3 \frac{\lambda_i^2 - \lambda_i(k_2 + k_3) + k_2k_3(1 - A_{23}A_{32})}{3\lambda_i^2 + 2s_1\lambda_i + s_2} e^{-\lambda_i t}$$

$$= \frac{\lambda_1^2 - \lambda_1(k_2 + k_3) + k_2k_3(1 - A_{23}A_{32})}{(\lambda_1 - \lambda_2)(\lambda_1 - \lambda_3)} e^{-\lambda_1 t}$$

$$+ \frac{\lambda_2^2 - \lambda_2(k_2 + k_3) + k_2k_3(1 - A_{23}A_{32})}{(\lambda_2 - \lambda_1)(\lambda_2 - \lambda_3)} e^{-\lambda_2 t}$$

$$+ \frac{\lambda_3^2 - \lambda_3(k_2 + k_3) + k_2k_3(1 - A_{23}A_{32})}{(\lambda_3 - \lambda_1)(\lambda_3 - \lambda_2)} e^{-\lambda_3 t}.$$

From the approximating equation

$$\frac{q_1}{q_0} = a_1 e^{-\lambda_1 t} + b_1 e^{-\lambda_2 t} + c_1 e^{-\lambda_3 t}$$

(here  $a_1$ ,  $b_1$  and  $c_1$  are coefficients of the normalized equation for the pool  $Q_1$ , where  $a_1 + b_1 + c_1 = 1$ ) five of the functions of the pool constants can be determined as follows: The coefficients  $s_1$ ,  $s_2$ , and  $s_3$  of  $D(p)$  are symmetric functions of  $\lambda$ 's:

$$s_1 = \lambda_1 + \lambda_2 + \lambda_3 = k_1 + k_2 + k_3$$

$$s_2 = \lambda_1\lambda_2 + \lambda_2\lambda_3 = \lambda_3\lambda_1 = k_1k_2(1 - A_{12}A_{21}) + k_2k_3(1 - A_{23}A_{32}) \\ + k_1k_3(1 - A_{13}A_{31})$$

$$s_3 = \lambda_1\lambda_2\lambda_3 = k_1k_2k_3(1 - A_{12}A_{21} - A_{23}A_{32} + A_{13}A_{31} - A_{12}A_{23}A_{31} - A_{13}A_{32}A_{21})$$

From

$$a_1 = \frac{\lambda_1^2 - \lambda_1(k_2 + k_3) + k_2k_3(1 - A_{23}A_{32})}{3\lambda_1^2 - 2s_1\lambda_1 + s_2}$$

and

$$b_1 = \frac{\lambda_2^2 - \lambda_2(k_2 + k_3) + k_2k_3(1 - A_{23}A_{32})}{3\lambda_2^2 - 2s_1\lambda_2 + s_2}$$

in conjunction with  $s_1$  and  $s_2$ , two additional constants are obtained, namely  $k_2 + k_3$  and  $k_2k_3(1 - A_{23}A_{32})$  (V.I.). After a slight simplification, the five independent functions of the pool constants, which can be obtained from the solution

$\frac{q_1}{q_0}$  become:

- (1)  $k_1$
- (2)  $k_2 + k_3$
- (3)  $k_2k_3(1 - A_{23}A_{32})$
- (4)  $k_2(1 - A_{12}A_{21}) + k_3(1 - A_{13}A_{31})$
- (5)  $k_2k_3(A_{12}A_{21} + A_{13}A_{31} + A_{12}A_{23}A_{31} + A_{13}A_{32}A_{21})$

When the system is in dynamic equilibrium and the quantity  $Q_1$  of the accessible pool is known, there remain six degrees of freedom (see Table I, Ref. 75). The number of arbitrary constants (degrees of freedom) may be reduced by two if the constants of the partial two-pool system can be determined separately, i.e., if  $\lambda_3$  and  $c_1$  are of significantly lower order of magnitude than  $\lambda_1$ ,  $\lambda_2$  and  $a_1$ ,  $b_1$  respectively. For instance, let  $Q_1$  and  $Q_2$  represent the initial two-pool system where  $Q_1$  is the accessible pool. Then, if  $k_1$ ,  $k_2$  and  $A_{12}A_{21}$  are determined separately as described in the preceding paper<sup>(75)</sup>, the available constants of the three-pool system become:

- (1)  $k_1$
- (2)  $k_2$
- (3)  $k_3$
- (4)  $A_{12}A_{21}$
- (5)  $A_{23}A_{32}$
- (6)  $A_{13}A_{31}$
- (7)  $A_{12}A_{23}A_{31} + A_{13}A_{32}A_{21}$

If, in addition,  $Q_1$  is known, the three-pool system (which is in dynamic equilibrium) has now four degrees of freedom, three of which may be expressed in terms

of modes of exit and entry, while the fourth can be given, for instance, either by the value of  $Q_3$  or by an  $A_{ij}$  (provided  $A_{ij}A_{ji} \neq 0$  or  $A_{ji} = 0$ ). Alternately, all four conditions can be expressed in terms of modes of exit and entry.

In general, once a particular model of a three-pool system has been decided upon, all the remaining pool constants can be calculated, though general formulations are no longer as simple as in the case of two-pool systems. When a sufficient number of acceptable constraints are imposed upon a system, the system becomes determinate (i.e., it has zero degrees of freedom) and all of its constants can be calculated from the approximating equation. We will first consider reduced three-pool "tracer distribution" systems, i.e., general reduced first order systems, without regard to the distribution of the basic non-tracer substance within the pools  $Q_1$ ,  $Q_2$  and  $Q_3$ , or to the corresponding equilibrium conditions. Such a general system is completely determined by the nine non-negative pool constants  $k_i$ 's and  $A_{ji}$ 's ( $i \neq j$ ;  $i = 1, 2, 3$ ;  $j = 0, 1, 2, 3$  and  $\sum_{\substack{j=0 \\ j \neq i}}^3 A_{ji} = 1$ )

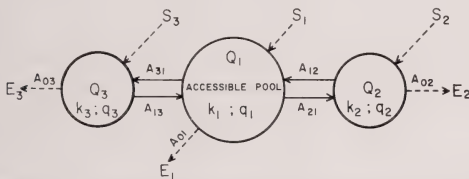


FIG. 2. Schematic representation of a three-pool system connected in series, with the accessible pool in the center.

(see Table II, Ref. 75). The exact values of these constants can then be derived from a given approximating equation and from an appropriate set of constraints (in terms of  $k_i$ 's and  $A_{ji}$ 's) which, together, completely characterize a particular model. In the case of a three-pool model in dynamic equilibrium (which is characterized by fifteen constants instead of the above nine) three further constraints (in addition to the three equilibrium conditions) must be imposed upon the system. The resulting additional calculations will be discussed at the end of this section.

In the following we will, at first, give explicit solutions for several determinate three-pool tracer distribution systems. We will consider only cases in which the "accessible" pool serves both as a receptacle for the initial tracer dose, as well as a sampling pool whose approximating tracer equation is a sum of three exponential functions. In cases where different pools are used for the introduction of the dose and for sampling, the general procedure for determination of pool constants remains the same, and explicit solutions can be obtained by similar methods.

Consider first a three-pool system connected in series (chainwise).

1. A three-pool system in series with the accessible pool  $Q_1$  in the center (Fig. 2).

$$\frac{q_i}{q_0} = a_i e^{\lambda_1 t} + b_i e^{\lambda_2 t} + c_i e^{\lambda_3 t}.$$

Here  $a_i$ ,  $b_i$  and  $c_i$  are coefficients of the normalized approximating equation for the  $i^{\text{th}}$  pool, i.e.,  $a_i + b_i + c_i = 1$  for  $i = 1, 2, 3$ .

$$\begin{aligned} D(p) &= \begin{vmatrix} p + k_1 & -A_{12}k_2 & -A_{13}k_3 \\ -A_{21}k_1 & p + k_2 & 0 \\ -A_{31}k_1 & 0 & p + k_3 \end{vmatrix} = (p + \lambda_1)(p + \lambda_2)(p + \lambda_3) \\ &= (p + k_1)(p + k_2)(p + k_3) - (p + k_2)A_{13}A_{31}k_1k_3 \\ &\quad - (p + k_3)A_{12}A_{21}k_1k_2 = p^3 + (k_1 + k_2 + k_3)p^2 + (k_1k_2 + k_2k_3 + k_3k_1 \\ &\quad - A_{12}A_{21}k_1k_2 - A_{13}A_{31}k_1k_3)p + k_1k_2k_3(1 - A_{12}A_{21} - A_{13}A_{31}) \\ &= p^3 + s_1p^2 + s_2p + s_3 = 0, \end{aligned}$$

where  $s_i$ 's are the symmetric functions of  $-\lambda_i$ 's:

$$s_1 = \lambda_1 + \lambda_2 + \lambda_3 = k_1 + k_2 + k_3.$$

$$s_2 = \lambda_1\lambda_2 + \lambda_2\lambda_3 + \lambda_3\lambda_1 = k_1k_2 + k_2k_3 + k_3k_1 - A_{12}A_{21}k_1k_2 - A_{13}A_{31}k_1k_3$$

$$s_3 = \lambda_1\lambda_2\lambda_3 = k_1k_2k_3(1 - A_{12}A_{21} - A_{13}A_{31}).$$

The coefficients  $a_1$ ,  $b_1$  and  $c_1$  can be expressed in terms of  $\lambda_i$ 's and pool constants as follows:

$$\begin{aligned} N_1(p) &= \begin{vmatrix} q_0 & -A_{12}k_2 & -A_{13}k_3 \\ 0 & p + k_2 & 0 \\ 0 & 0 & p + k_3 \end{vmatrix} = q_0(p + k_2)(p + k_3) \\ &= q_0[k_2k_3 + (k_2 + k_3)p + p^2]. \end{aligned}$$

Hence,

$$\begin{aligned} a_1 &= \frac{N_1(-\lambda_1)}{q_0 D'(-\lambda_1)} = \frac{k_2k_3 - (k_2 + k_3)\lambda_1 + \lambda_1^2}{(\lambda_1 - \lambda_2)(\lambda_1 - \lambda_3)}, \\ b_1 &= \frac{N_1(-\lambda_2)}{q_0 D'(-\lambda_2)} = -\frac{k_2k_3 - (k_2 + k_3)\lambda_2 + \lambda_2^2}{(\lambda_1 - \lambda_2)(\lambda_2 - \lambda_3)}, \\ c_1 &= \frac{N_1(-\lambda_3)}{q_0 D'(-\lambda_3)} = \frac{k_2k_3 - (k_2 + k_3)\lambda_3 + \lambda_3^2}{(\lambda_1 - \lambda_3)(\lambda_2 - \lambda_3)}, \end{aligned}$$

where  $a_1 + b_1 + c_1 = 1$ .

Clearing fractions for  $a_1$  and  $b_1$  gives:

$$(1) \quad k_2k_3 - (k_2 + k_3)\lambda_1 = a_1(\lambda_1 - \lambda_2)(\lambda_1 - \lambda_3) - \lambda_1^2$$

$$(2) \quad k_2k_3 - (k_2 + k_3)\lambda_2 = -b_1(\lambda_1 - \lambda_2)(\lambda_2 - \lambda_3) - \lambda_2^2.$$

Subtracting (2) from (1) and dividing by  $(\lambda_2 - \lambda_1)$ :



$$\begin{aligned}
 k_2 + k_3 &= -a_1(\lambda_1 - \lambda_3) - b_1(\lambda_2 - \lambda_3) + \lambda_1 + \lambda_2 \\
 &= a_1(\lambda_2 + \lambda_3) + b_1(\lambda_1 + \lambda_3) + (\lambda_1 + \lambda_2)(1 - a_1 - b_1)
 \end{aligned}$$

or

$$k_2 + k_3 = a_1(\lambda_2 + \lambda_3) + b_1(\lambda_1 + \lambda_3) + c_1(\lambda_1 + \lambda_2).$$

Multiplying (1) and (2) by  $\lambda_2$  and  $\lambda_1$  respectively, one gets:

$$\begin{aligned}
 k_2 k_3 \lambda_2 - (k_2 + k_3) \lambda_1 \lambda_2 &= a_1(\lambda_1 - \lambda_2)(\lambda_1 - \lambda_3) \lambda_2 - \lambda_1^2 \lambda_2 \\
 k_2 k_3 \lambda_1 - (k_2 + k_3) \lambda_1 \lambda_2 &= -b_1(\lambda_1 - \lambda_2)(\lambda_2 - \lambda_3) \lambda_1 - \lambda_1 \lambda_2^2.
 \end{aligned}$$

Subtracting, and dividing by  $(\lambda_2 - \lambda_1)$ :

$$\begin{aligned}
 k_2 k_3 &= -a_1(\lambda_1 - \lambda_3) \lambda_2 - a_1(\lambda_2 - \lambda_3) \lambda_1 + \lambda_1 \lambda_2 \\
 &= a_1 \lambda_2 \lambda_3 + b_1 \lambda_1 \lambda_3 + (1 - a - b) \lambda_1 \lambda_2
 \end{aligned}$$

or

$$k_2 k_3 = a_1 \lambda_2 \lambda_3 + b_1 \lambda_1 \lambda_3 + c_1 \lambda_1 \lambda_2.$$

Hence  $k_1 = s_1 - (k_2 + k_3)$ , and  $k_2$  and  $k_3$  can be calculated from  $k_2 + k_3$  and  $k_2 k_3$ , though which is larger (i.e.,  $k_2$  or  $k_3$ ) will depend on other data than those derived from the approximating equation. The values for  $\frac{q_2}{q_0}$  (and hence by symmetry for  $\frac{q_3}{q_0}$ ) can be obtained as above:

$$N_2(p) = \begin{vmatrix} p + k_1 & q_0 & -A_{13}k_3 \\ -A_{21}k_1 & 0 & 0 \\ -A_{31}k_1 & 0 & p + k_3 \end{vmatrix} = q_0 A_{21} k_1 (p + k_3)$$

and

$$\begin{aligned}
 \frac{q_2}{q_0} &= A_{21} k_1 \left[ \frac{k_3 - \lambda_1}{(\lambda_1 - \lambda_2)(\lambda_1 - \lambda_3)} e^{-\lambda_1 t} \right. \\
 &\quad \left. - \frac{k_3 - \lambda_2}{(\lambda_1 - \lambda_2)(\lambda_2 - \lambda_3)} e^{-\lambda_2 t} + \frac{k_3 - \lambda_3}{(\lambda_1 - \lambda_3)(\lambda_2 - \lambda_3)} e^{-\lambda_3 t} \right].
 \end{aligned}$$

Case 1: Let  $A_{03} = 0$  (i.e.,  $A_{13} = 1$  and hence  $A_{31} = A_{13}A_{31}$ ). (Fig. 3). From the above,  $k_1$ ,  $k_2$  and  $k_3$  are known in addition to  $a_1$ ,  $b_1$ , and  $c_1$ , and  $\lambda_1$ ,  $\lambda_2$  and  $\lambda_3$ .

Also

$$s_3 = \lambda_1 \lambda_2 \lambda_3 = k_1 k_2 k_3 - A_{12} A_{21} k_1 k_2 k_3 - A_{31} k_1 k_2 k_3$$

and

$$-s_2 k_3 = -k_1 k_2 k_3 + A_{12} A_{21} k_1 k_2 k_3 + A_{31} k_1 k_3^2 - k_2 k_3^2 - k_1 k_3^2.$$

Adding the two expressions one gets

$$s_3 - s_2 k_3 = A_{31}(k_1 k_1^2 - k_1 k_2 k_3) - k_2 k_3^2 - k_1 k_3^2$$

or

$$A_{31} = A_{13} A_{31} = \frac{s_3 - s_2 k_3 + k_3^2(k_1 + k_2)}{k_1 k_3(k_3 - k_2)} = \frac{s_2 k_3 - s_3 - k_3^2(k_1 + k_2)}{k_1 k_3(k_2 - k_3)}$$

Similarly:

$$s_3 = k_1 k_2 k_3 - A_{12} A_{21} k_1 k_2 k_3 - A_{31} k_1 k_2 k_3$$

$$-k_2 s_2 = -k_1 k_2 k_3 + A_{12} A_{21} k_1 k_2^2 + A_{31} k_1 k_2 k_3 - k_2^2 k_3 - k_1 k_2^2$$

$$s_3 - k_2 s_2 = A_{12} A_{21} k_1 k_2(k_2 - k_3) - k_2^2(k_1 + k_3)$$

and hence

$$A_{12} A_{21} = \frac{s_3 - k_2 s_2 + k_2^2(k_1 + k_3)}{k_1 k_2(k_2 - k_3)}$$

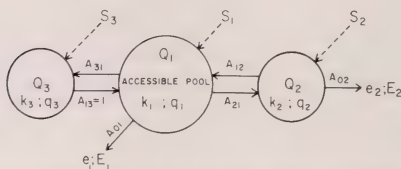


FIG. 3. Schematic representation of a three-pool system connected in series, with the accessible pool in the center, and  $A_{03} = 0$ .

As previously defined,  $e_1$  = fractional activity eventually excreted through  $Q_1$ :

$$\begin{aligned} c_1 &= \int_0^\infty k_1 A_{01}(t) a_1 e^{-\lambda_1 t} + b_1 e^{-\lambda_2 t} + c_1 e^{-\lambda_3 t} dt \\ &= k_1 A_{01} \left[ \frac{a_1}{\lambda_1} + \frac{b_1}{\lambda_2} + \frac{c_1}{\lambda_3} \right] \end{aligned}$$

and

$$A_{01} = \frac{e_1}{k_1 \left[ \frac{a_1}{\lambda_1} + \frac{b_1}{\lambda_2} + \frac{c_1}{\lambda_3} \right]}$$

$$A_{21} = 1 - A_{31} - A_{11}$$

$$A_{12} = \frac{A_{12} A_{21}}{A_{21}}$$

$$A_{02} = 1 - A_{12}$$

Case 2. Nothing leaves the system through pool  $Q_1$ . Let  $e_2$  = fraction of tracer leaving through pool  $Q_2$  (Fig. 4).

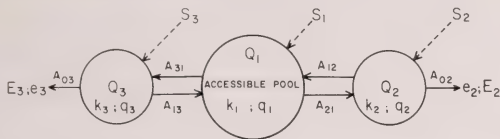


FIG. 4. Schematic representation of a three-pool system connected in series, with the accessible pool in the center, and  $A_{01} = 0$ .

As in Case 1.

$$A_{12}A_{21} = \frac{s_3 - k_2s_2 + k_2^2(k_1 + k_3)}{k_1k_2(k_2 - k_3)}$$

and

$$A_{13}A_{31} = \frac{s_2k_3 - s_3 - k_3^2(k_1 + k_2)}{k_1k_3(k_2 - k_3)}$$

The tracer activity in  $Q_2$  is

$$q_2 = q_0 \sum_1^3 \frac{N_2(-\lambda_i)}{D'(-\lambda_i)} e^{-\lambda_i t},$$

where

$$N_2(p) = \begin{vmatrix} p + k_1 & q_0 & -A_{13}k_3 \\ -A_{21}k_1 & 0 & 0 \\ -A_{31}k_1 & 0 & p + k_3 \end{vmatrix} = q_0 A_{21}k_1(p + k_3).$$

Hence

$$\frac{q_2}{q_0} = A_{21}k_1 \left( -\frac{\lambda_1 - k_3}{(\lambda_1 - \lambda_2)(\lambda_1 - \lambda_3)} e^{-\lambda_1 t} + \frac{\lambda_2 - k_3}{(\lambda_1 - \lambda_2)(\lambda_2 - \lambda_3)} e^{-\lambda_2 t} + \frac{k_3 - \lambda_3}{(\lambda_1 - \lambda_3)(\lambda_2 - \lambda_3)} e^{-\lambda_3 t} \right)$$

and

$$\begin{aligned} e_2 &= k_2 A_{02} \int_0^\infty \frac{q_2}{q_0} dt = A_{21} A_{02} k_1 k_2 \left[ -\frac{\lambda_1 - k_3}{(\lambda_1 - \lambda_2)(\lambda_1 - \lambda_3)\lambda_1} \right. \\ &\quad \left. + \frac{\lambda_2 - k_3}{(\lambda_1 - \lambda_2)(\lambda_2 - \lambda_3)\lambda_2} + \frac{k_3 - \lambda_3}{(\lambda_1 - \lambda_3)(\lambda_2 - \lambda_3)\lambda_3} \right] \\ &= A_{21} A_{02} k_1 k_2 \left[ \frac{(k_3 - \lambda_1)(\lambda_2 - \lambda_3)\lambda_2\lambda_3}{\lambda_1\lambda_2\lambda_3(\lambda_1 - \lambda_2)(\lambda_1 - \lambda_3)(\lambda_2 - \lambda_3)} \right] \end{aligned}$$

$$+ \frac{(\lambda_2 - k_3)(\lambda_1 - \lambda_3)\lambda_1\lambda_2 + (k_3 - \lambda_3)(\lambda_1 - \lambda_2)\lambda_1\lambda_2}{\lambda_1\lambda_2\lambda_3(\lambda_1 - \lambda_2)(\lambda_1 - \lambda_3)(\lambda_2 - \lambda_3)} \Big] \\ e_2 = \frac{A_{21}A_{02}k_1k_2k_3}{\lambda_1\lambda_2\lambda_3} = \frac{A_{21}A_{02}k_1k_2k_3}{s_3}.$$

Since  $A_{12} = 1 - A_{02}$  we get

$$A_{21} = \frac{s_3 - k_2s_2 + k_2^2(k_1 + k_3)}{k_1k_2(k_2 - k_3)} \frac{1}{A_{12}} = X \frac{1}{A_{12}} = \frac{X}{1 - A_{02}} = \frac{A_{12}A_{21}}{A_{12}}.$$

Also

$$A_{21} = \frac{e_2s_3}{k_1k_2k_3} \frac{1}{A_{02}} = Y \frac{1}{A_{02}}.$$

Hence

$$\frac{X}{1 - A_{02}} = \frac{Y}{A_{02}} \quad \text{or} \quad A_{02}X = (1 - A_{02})Y,$$

and

$$A_{02} = \frac{Y}{X + Y};$$

therefore:

$$A_{21} = \frac{Y}{A_{02}} = X + Y = \frac{s_3 - k_2s_2 + k_2^2(k_1 + k_3)}{k_1k_2(k_2 - k_3)} + \frac{e_2s_3}{k_1k_2k_3} = A_{12}A_{21} + \frac{e_2s_3}{k_1k_2k_3}$$

$$A_{12} = \frac{A_{12}A_{21}}{A_{21}}$$

$$A_{31} = 1 - A_{21}$$

$$A_{13} = \frac{A_{13}A_{31}}{A_{31}}$$

$$A_{02} = 1 - A_{12}$$

$$A_{03} = 1 - A_{13}.$$

Or alternately,

$$e_1 = 1 - e_2 = \frac{A_{31}A_{03}k_1k_2k_3}{s_3};$$

$$A_{03} = \frac{(1 - e_2)s_3}{k_1k_2k_3A_{31}}; \quad A_{13} = 1 - A_{03};$$

$$A_{02} = \frac{Y}{A_{21}} = \frac{e_2s_3}{k_1k_2k_3A_{21}}; \quad A_{12} = 1 - A_{02}.$$

II. A three-pool system in series, with the accessible pool  $Q_2$  at one end (Fig. 5).

$$D(p) = \begin{vmatrix} p + k_1 & -A_{12}k_2 & -A_{13}k_3 \\ -A_{21}k_1 & p + k_2 & 0 \\ -A_{31}k_1 & 0 & p + k_3 \end{vmatrix} = (p + \lambda_1)(p + \lambda_2)(p + \lambda_3) = 0,$$

where, as before,

$$s_1 = \lambda_1 + \lambda_2 + \lambda_3 = k_1 + k_2 + k_3$$

$$s_2 = \lambda_1\lambda_2 + \lambda_2\lambda_3 + \lambda_3\lambda_1 = k_1k_2 + k_2k_3 + k_3k_1 - A_{12}A_{21}k_1k_2 - A_{13}A_{31}k_1k_3$$

$$s_3 = \lambda_1\lambda_2\lambda_3 = k_1k_2k_3(1 - A_{12}A_{21} - A_{13}A_{31}).$$

Also:

$$N_2(p) = \begin{vmatrix} p + k_1 & 0 & -A_{13}k_3 \\ -A_{21}k_1 & q_0 & 0 \\ -A_{31}k_1 & 0 & p + k_3 \end{vmatrix} = q_0(p + k_1)(p + k_3) - q_0A_{13}A_{31}k_1k_3$$

$$= q_0[p^2 + (k_1 + k_3)p + k_1k_3(1 - A_{13}A_{31})].$$

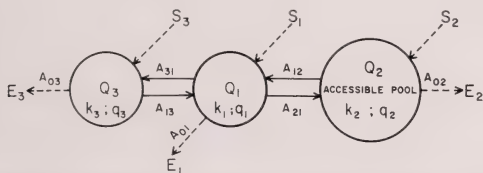


FIG. 5. Schematic representation of a three-pool system connected in series, with the accessible pool at one end of the series.

Hence:

$$\begin{aligned} \frac{q_2}{q_0} &= \frac{k_1k_3(1 - A_{13}A_{31}) - (k_1 + k_3)\lambda_1 + \lambda_1^2}{(\lambda_1 - \lambda_2)(\lambda_1 - \lambda_3)} e^{-\lambda_1 t} \\ &\quad - \frac{k_1k_3(1 - A_{13}A_{31}) - (k_1 + k_3)\lambda_2 + \lambda_2^2}{(\lambda_1 - \lambda_2)(\lambda_2 - \lambda_3)} e^{-\lambda_2 t} \\ &\quad + \frac{k_1k_3(1 - A_{13}A_{31}) - (k_1 + k_3)\lambda_3 + \lambda_3^2}{(\lambda_1 - \lambda_3)(\lambda_2 - \lambda_3)} e^{-\lambda_3 t} \\ &= a_2 e^{-\lambda_1 t} + b_2 e^{-\lambda_2 t} + c_2 e^{-\lambda_3 t}, \end{aligned}$$

and

$$k_1k_3(1 - A_{13}A_{31}) - (k_1 + k_3)\lambda_1 = a_2(\lambda_1 - \lambda_2)(\lambda_1 - \lambda_3) - \lambda_1^2$$

$$k_1k_3(1 - A_{13}A_{31}) - (k_1 + k_3)\lambda_2 = -b_2(\lambda_1 - \lambda_2)(\lambda_2 - \lambda_3) - \lambda_2^2.$$

Subtracting and dividing by  $\lambda_2 - \lambda_1$  we then get:

$$\begin{aligned} k_1 + k_3 &= a_2(\lambda_3 - \lambda_1) + b_2(\lambda_3 - \lambda_2) + \lambda_1 + \lambda_2 \\ &= a_2(\lambda_2 + \lambda_3) + b_2(\lambda_1 + \lambda_3) + (1 - a_2 - b_2)(\lambda_1 + \lambda_2) \end{aligned}$$

or

$$k_1 + k_3 = a_2(\lambda_2 + \lambda_3) + b_2(\lambda_1 + \lambda_3) + c_2(\lambda_1 + \lambda_2) = X_1.$$

Multiplying the above equations by  $\lambda_2$  and  $\lambda_1$  respectively:

$$\begin{aligned} k_1 k_3 \lambda_2 - (k_1 + k_3) \lambda_1 \lambda_2 &= a_2(\lambda_1 - \lambda_2)(\lambda_1 - \lambda_3) \lambda_2 - \lambda_1^2 \lambda_2 + A_{13} A_{31} k_1 k_3 \lambda_2 \\ k_1 k_3 \lambda_1 - (k_1 + k_3) \lambda_1 \lambda_2 &= -b_2(\lambda_1 - \lambda_2)(\lambda_2 - \lambda_3) \lambda_1 - \lambda_1 \lambda_2^2 + A_{13} A_{31} k_1 k_3 \lambda_1. \end{aligned}$$

Subtracting and dividing by  $\lambda_2 - \lambda_1$ :

$$\begin{aligned} k_1 k_3 &= a_2(\lambda_3 - \lambda_1) \lambda_2 + b_2(\lambda_3 - \lambda_2) \lambda_1 + \lambda_1 \lambda_2 + A_{13} A_{31} k_1 k_3 \\ &= a_2(\lambda_2 \lambda_3 - \lambda_1 \lambda_2) + b_2(\lambda_1 \lambda_3 - \lambda_1 \lambda_2) + \lambda_1 \lambda_2 + A_{13} A_{31} k_1 k_3 \\ &= a_2 \lambda_2 \lambda_3 + b_2 \lambda_1 \lambda_3 + \lambda_1 \lambda_2 (1 - a_2 - b_2) + A_{13} A_{31} k_1 k_3. \\ k_1 k_3 &= a_2 \lambda_2 \lambda_3 + b_2 \lambda_1 \lambda_3 + c_2 \lambda_1 \lambda_2 + A_{13} A_{31} k_1 k_3 = X_2 + A_{13} A_{31} k_1 k_3 \end{aligned}$$

or

$$k_1 k_3 = \frac{X_2}{1 - A_{13} A_{31}}.$$

Since here, as before,

$$A_{12} A_{21} = \frac{s_3 - s_2 k_2 - k_2^2 (k_1 + k_3)}{k_1 k_2 (k_2 - k_3)}$$

and

$$A_{13} A_{31} = \frac{s_2 k_3 - s_3 - k_3^2 (k_1 + k_2)}{k_1 k_3 (k_2 - k_3)}$$

one gets

$$k_1 k_3 = X_2 + \frac{s_2 k_3 - s_3 - k_3^2 (k_1 + k_2)}{(k_2 - k_3)}.$$

Using  $k_1 + k_3 = X_1$  (i.e.,  $k_1 = X_1 - k_3$ ) and  $k_2 = s_1 - (k_1 + k_3) = s_1 - X_1$ , the above equation can be solved for  $k_3$ :

$$k_3 = \frac{X_2 s_1 - X_1 X_2 - s_3}{X_1 s_1 + X_2 - X_1^2 - s_2}.$$

The values of  $k_1$  and  $k_2$  follow from

$$k_1 = X_1 - k_3$$

and

$$k_2 = s_1 - (k_1 + k_3) = s_1 - X_1.$$

It should be noted that in this case  $k_1$  and  $k_3$  are unequivocally determined by



the above solution for  $k_3$ . The values for  $\frac{q_1}{q_0}$  and  $\frac{q_3}{q_0}$  can be obtained in the usual way:

$$N_1(p) = \begin{vmatrix} 0 & -A_{12}k_2 & -A_{13}k_3 \\ q_0 & p + k_2 & 0 \\ 0 & 0 & p + k_3 \end{vmatrix} = q_0 A_{12}k_2(p + k_3),$$

$$\frac{q_1}{q_0} = A_{12}k_2 \left[ \frac{k_3 - \lambda_1}{(\lambda_1 - \lambda_2)(\lambda_1 - \lambda_3)} e^{-\lambda_1 t} - \frac{k_3 - \lambda_2}{(\lambda_1 - \lambda_2)(\lambda_2 - \lambda_3)} e^{-\lambda_2 t} + \frac{k_3 - \lambda_3}{(\lambda_1 - \lambda_3)(\lambda_2 - \lambda_3)} e^{-\lambda_3 t} \right].$$

$$N_3(p) = \begin{vmatrix} p + k_1 & -A_{12}k_2 & 0 \\ -A_{21}k_1 & p + k_2 & q_0 \\ -A_{31}k_1 & 0 & 0 \end{vmatrix} = q_0 A_{12}A_{31}k_1k_2,$$

$$\frac{q_3}{q_0} = A_{12}A_{31}k_1k_2 \left[ \frac{e^{-\lambda_1 t}}{(\lambda_1 - \lambda_2)(\lambda_1 - \lambda_3)} - \frac{e^{-\lambda_2 t}}{(\lambda_1 - \lambda_2)(\lambda_2 - \lambda_3)} + \frac{e^{-\lambda_3 t}}{(\lambda_1 - \lambda_3)(\lambda_2 - \lambda_3)} \right]$$

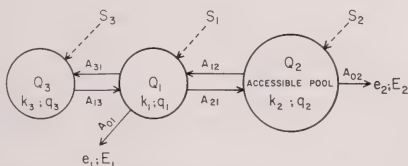


FIG. 6. Schematic representation of a three-pool system connected in series, with the accessible pool at one end of the series, and  $A_{03} = 0$ .

Case 1.  $A_{03} = 0$ ;  $e_2$  = fraction of total activity excreted through the accessible pool  $Q_2$  (Fig. 6).

Then

$$e_2 = k_2 A_{02} \int_0^{\infty} (a_2 e^{-\lambda_1 t} + b_2 e^{-\lambda_2 t} + c_2 e^{-\lambda_3 t}) dt$$

$$e_2 = k_2 A_{02} \left( \frac{a_2}{\lambda_1} + \frac{b_2}{\lambda_2} + \frac{c_2}{\lambda_3} \right)$$

and

$$A_{02} = \frac{e_2}{k_2 \left( \frac{a_2}{\lambda_1} + \frac{b_2}{\lambda_2} + \frac{c_2}{\lambda_3} \right)}.$$

$$A_{12} = 1 - A_{02}$$

$$A_{21} = \frac{A_{12}A_{21}}{A_{12}}$$

$$A_{13} = 1, \quad (\text{by hypothesis})$$

$$A_{31} = A_{13}A_{31}$$

$$A_{01} = 1 - A_{21} - A_{31}.$$

Case 2.  $A_{01} = 0$ ;  $e_2$  given. Then as before (Fig. 7):

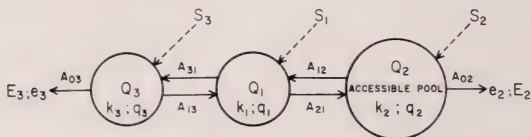


FIG. 7. Schematic representation of a three-pool system connected in series, with the accessible pool at one end of the series, and  $A_{01} = 0$ .

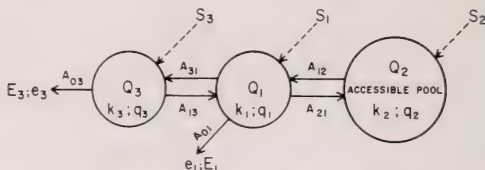


FIG. 8. Schematic representation of a three-pool system connected in series, with the accessible pool at one end of the series, and  $A_{02} = 0$ .

$$A_{02} = \frac{e_2}{k_2 \left( \frac{a_2}{\lambda_1} + \frac{b_2}{\lambda_2} + \frac{c_2}{\lambda_3} \right)}$$

$$A_{12} = 1 - A_{02}$$

$$A_{21} = \frac{A_{12}A_{21}}{A_{12}}$$

$$A_{31} = 1 - A_{21}$$

$$A_{13} = \frac{A_{13}A_{31}}{A_{31}}$$

$$A_{03} = 1 - A_{13}.$$

Case 3.  $A_{02} = 0$ ;  $e_1$  = fraction of total activity excreted through Q<sub>1</sub> (Fig. 8):

$$e_1 = k_1 A_{01} \int_0^\infty \left\{ \frac{A_{12} k_2 (k_3 - \lambda_1)}{(\lambda_1 - \lambda_2)(\lambda_1 - \lambda_3)} e^{-\lambda_1 t} - \frac{A_{12} k_2 (k_3 - \lambda_2)}{(\lambda_1 - \lambda_2)(\lambda_2 - \lambda_3)} e^{-\lambda_2 t} \right.$$

$$+ \frac{A_{12}k_2(k_3 - \lambda_3)}{(\lambda_1 - \lambda_3)(\lambda_2 - \lambda_3)} e^{-\lambda_3 t} \Big\} dt = A_{01}A_{12}k_1k_2 \left[ \frac{k_3 - \lambda_1}{(\lambda_1 - \lambda_2)(\lambda_1 - \lambda_3)\lambda_1} - \frac{k_3 - \lambda_2}{(\lambda_1 - \lambda_2)(\lambda_2 - \lambda_3)\lambda_2} + \frac{k_3 - \lambda_3}{(\lambda_1 - \lambda_3)(\lambda_2 - \lambda_3)\lambda_3} \right] = \frac{A_{01}A_{12}k_1k_2k_3}{s_3}.$$

$$A_{12} = 1, \quad (\text{by hypothesis})$$

$$A_{01} = \frac{e_1 s_3}{k_1 k_2 k_3}$$

$$A_{21} = A_{12}A_{21} = \frac{s_3 - k_2 s_2 + k_2^2(k_1 + k_3)}{k_1 k_2 (k_2 - k_3)}$$

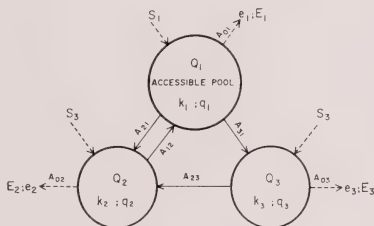
$$A_{31} = 1 - A_{21} - A_{01}$$

$$A_{13} = \frac{A_{13}A_{31}}{A_{31}}$$

$$A_{03} = 1 - A_{13}.$$

III. A cyclic three-pool system with the accessible pool  $Q_1$  and  $A_{13} = A_{22} = 0$  (Fig. 9):

FIG. 9. Schematic representation of a cyclic three-pool system with accessible pool  $Q_1$ , with bilateral interchange between pools  $Q_1$  and  $Q_2$ , and with unidirectional transfers from pool  $Q_1$  to pool  $Q_3$ , and from pool  $Q_3$  to pool  $Q_2$ .



$$\frac{q_1}{q_0} = a_1 e^{-\lambda_1 t} + b_1 e^{-\lambda_2 t} + c_1 e^{-\lambda_3 t}.$$

$$D(p) = \begin{vmatrix} p + k_1 & -A_{12}k_2 & 0 \\ -A_{21}k_1 & p + k_2 & -A_{23}k_3 \\ -A_{31}k_1 & 0 & p + k_3 \end{vmatrix}$$

$$\begin{aligned} &= (p + k_1)(p + k_2)(p + k_3) - A_{31}A_{12}A_{23}k_1k_2k_3 - A_{12}A_{21}k_1k_2(p + k_3) \\ &= p^3 + p^2(k_1 + k_2 + k_3) + p(k_1k_2 + k_2k_3 + k_3k_1 - A_{12}A_{21}k_1k_2) \\ &\quad + k_1k_2k_3(1 - A_{12}A_{23}A_{31} - A_{12}A_{21}) = 0. \end{aligned}$$

$$s_1 = \lambda_1 + \lambda_2 + \lambda_3 = k_1 + k_2 + k_3$$

$$s_2 = \lambda_1\lambda_2 + \lambda_2\lambda_3 + \lambda_3\lambda_1 = k_1k_2 + k_2k_3 + k_3k_1 - A_{12}A_{21}k_1k_2$$

$$s_3 = \lambda_1 \lambda_2 \lambda_3 = k_1 k_2 k_3 (1 - A_{12} A_{23} A_{31} - A_{12} A_{21})$$

$$A_{12} A_{21} = \frac{k_1 k_2 + k_2 k_3 + k_3 k_1 - s_2}{k_1 k_2}$$

$$A_{12} A_{23} A_{31} = 1 - A_{12} A_{21} - \frac{s_3}{k_1 k_2 k_3} = \frac{k_3 s_2 - s_3 - k_3^2 (k_1 + k_2)}{k_1 k_2 k_3}$$

$$N_1(p) = \begin{vmatrix} q_0 & -A_{12}k_2 & 0 \\ 0 & p + k_2 & -A_{23}k_3 \\ 0 & 0 & p + k_3 \end{vmatrix} = q_0(p + k_2)(p + k_3) \\ = q_0[p^2 + p(k_2 + k_3) + k_2 k_3]$$

This gives the same expressions for  $a_1$ ,  $b_1$  and  $c_1$  as in I (p. 240) and hence:

$$k_2 + k_3 = a_1(\lambda_2 + \lambda_3) + b_1(\lambda_1 + \lambda_3) + c_1(\lambda_1 + \lambda_2)$$

$$k_2 k_3 = a_1 \lambda_2 \lambda_3 + b_1 \lambda_1 \lambda_3 + c_1 \lambda_1 \lambda_2$$

$$k_1 = s_1 - (k_2 + k_3).$$

Here, as in I,  $k_2$  and  $k_3$  cannot be differentiated on the basis of the solution for  $\frac{q_1}{q_0}$  alone. The solutions for  $\frac{q_2}{q_0}$  and  $\frac{q_3}{q_0}$  are obtained as before:

$$N_2(p) = \begin{vmatrix} p + k_1 & q_0 & 0 \\ -A_{21}k_1 & 0 & -A_{23}k_3 \\ -A_{31}k_1 & 0 & p + k_3 \end{vmatrix} = q_0[A_{21}k_1(p + k_3) + A_{23}A_{31}k_1k_3]$$

$$\frac{q_2}{q_0} = \frac{A_{21}k_1(k_3 - \lambda_1) + A_{23}A_{31}k_1k_3}{(\lambda_1 - \lambda_2)(\lambda_1 - \lambda_3)} e^{-\lambda_1 t} - \frac{A_{21}k_1(k_3 - \lambda_2) + A_{23}A_{31}k_1k_3}{(\lambda_1 - \lambda_2)(\lambda_2 - \lambda_3)} e^{-\lambda_2 t} \\ + \frac{A_{21}k_1(k_3 - \lambda_3) + A_{23}A_{31}k_1k_3}{(\lambda_1 - \lambda_3)(\lambda_2 - \lambda_3)} e^{-\lambda_3 t}.$$

$$N_3(p) = \begin{vmatrix} p + k_1 & -A_{12}k_2 & q_0 \\ -A_{21}k_1 & p + k_2 & 0 \\ -A_{31}k_1 & 0 & 0 \end{vmatrix} = q_0(p + k_2)A_{31}k_1$$

$$\frac{q_3}{q_0} = \frac{A_{31}k_1(k_2 - \lambda_1)}{(\lambda_1 - \lambda_2)(\lambda_1 - \lambda_3)} e^{-\lambda_1 t} - \frac{A_{31}k_1(k_2 - \lambda_2)}{(\lambda_1 - \lambda_2)(\lambda_2 - \lambda_3)} e^{-\lambda_2 t} \\ + \frac{A_{31}k_1(k_2 - \lambda_3)}{(\lambda_1 - \lambda_3)(\lambda_2 - \lambda_3)} e^{-\lambda_3 t}.$$

Consider the most general case where  $c_1$  and  $c_3$  are given, such that  $c_1 + c_3 \leq 1$ , then

$$c_1 = k_1 A_{31} \int_0^\infty (a_1 e^{-\lambda_1 t} + b_1 e^{-\lambda_2 t} + c_1 e^{-\lambda_3 t}) dt,$$

and therefore

$$A_{01} = \frac{c_1}{k_1 \left( \frac{a_1}{\lambda_1} + \frac{b_1}{\lambda_2} + \frac{c_1}{\lambda_3} \right)}$$

From the equation for  $\frac{q_3}{q_0}$  it follows that

$$\begin{aligned} c_3 &= k_3 A_{03} \int_0^\infty \frac{q_3}{q_0} dt = k_1 k_3 A_{03} A_{31} \left[ \frac{k_2 - \lambda_1}{(\lambda_1 - \lambda_2)(\lambda_1 - \lambda_3)} \right. \\ &\quad \left. - \frac{k_2 - \lambda_2}{(\lambda_1 - \lambda_2)(\lambda_2 - \lambda_3)} + \frac{k_2 - \lambda_3}{(\lambda_1 - \lambda_3)(\lambda_2 - \lambda_3)} \right] \\ &= \frac{A_{03} A_{31} k_1 k_2 k_3}{\lambda_1 \lambda_2 \lambda_3} = \frac{A_{03} A_{31} k_1 k_2 k_3}{s_3} = \frac{A_{03} A_{31}}{1 - A_{12} A_{23} A_{31} - A_{12} A_{21}} \\ A_{03} A_{31} &= \frac{e_3 s_3}{k_1 k_2 k_3}. \end{aligned}$$

Let

$$(1) \quad A_{12} A_{21} = X \quad \text{or} \quad A_{12} = \frac{X}{A_{21}},$$

$$(2) \quad A_{12} A_{23} A_{31} = Y,$$

and

$$(3) \quad A_{03} A_{31} = Z.$$

Also:

$$(4) \quad A_{21} + A_{31} + A_{01} = 1; \quad A_{31} = 1 - A_{21} - A_{01}$$

$$(5) \quad A_{03} + A_{23} = 1; \quad A_{23} = 1 - A_{03}$$

$$(6) \quad A_{12} + A_{02} = 1.$$

Substituting the values for (1), (4) and (5) into equation (2):

$$(7) \quad \frac{X}{A_{21}} (1 - A_{03})(1 - A_{21} - A_{01}) = Y.$$

Substituting the value of  $A_{31}$  from (4) into (3):

$$A_{03}(1 - A_{21} - A_{01}) = Z \quad \text{or} \quad A_{03} = \frac{Z}{1 - A_{21} - A_{01}},$$

and finally, substituting this value for  $A_{03}$  into (7):

$$\frac{X}{A_{21}} \left( 1 - \frac{Z}{1 - A_{21} - A_{01}} \right) (1 - A_{21} - A_{01}) = \frac{X}{A_{21}} (1 - A_{21} - A_{01} - Z) = Y$$

or

$$A_{21} = \frac{X}{X + Y} (1 - A_{01} - Z) = \frac{A_{12}A_{21}(1 - A_{01} - A_{03}A_{31})}{A_{12}A_{21} + A_{12}A_{23}A_{31}}$$

$$= \frac{k_1k_2k_3 + k_3^2(k_1 + k_2) - s_2k_3}{k_1k_2k_3 - s_3} \left( 1 - A_{01} - \frac{e_3s_3}{k_1k_2k_3} \right).$$

$$A_{12} = \frac{X}{A_{21}} = \frac{A_{12}A_{21}}{A_{21}}$$

$$A_{31} = 1 - A_{21} - A_{01}; \quad \left( A_{01} = \frac{e_1}{k_1 \left( \frac{a_1}{\lambda_1} + \frac{b_1}{\lambda_2} + \frac{c_1}{\lambda_3} \right)} \right)$$

$$A_{13} = A_{32} = 0 \quad (\text{by hypothesis})$$

$$A_{03} = \frac{A_{03}A_{31}}{A_{31}} = \frac{Z}{A_{31}} = \frac{e_3s_3}{k_1k_2k_3A_{31}}.$$

$$A_{23} = 1 - A_{03}$$

$$A_{02} = 1 - A_{12}.$$

Similarly, the constants  $k_i$ 's and  $A_{ji}$ 's can be determined when  $Q_2$  or  $Q_3$  in Figure 9 is the accessible pool.

Consider now a three-pool system in dynamic equilibrium whose constants  $k_i$ 's and  $A_{ji}$ 's have been determined for the corresponding "tracer distribution" system. Such a steady-state system can be completely characterized by imposing upon it three additional constraints, at least one of which is expressed in terms of the amounts of basic substance either present in a given pool (or a combination of pools), or leaving or entering the same. As an example we will consider Case 1 of a three-pool system in series with the accessible pool in the center (Fig. 3).

(a) Let the three constraints be: 1) amount  $Q_1$  of the basic substance in pool  $Q_1$ , 2)  $S_2 = 0$ , and 3)  $S_3 = 0$ . Then, using equilibrium conditions we get

$$Q_2 = \frac{A_{21}k_1Q_1}{k_2}; \quad Q_3 = \frac{A_{31}k_1Q_1}{k_3}.$$

$$E_1 = A_{01}k_1Q_1$$

$$E_2 = A_{02}k_2Q_2 = A_{02}A_{21}k_1Q_1$$

$$S_1 = k_1Q_1(1 - A_{31}A_{12}A_{21}) = E_1 + E_2.$$

(b) Let  $S_1$ ,  $S_2$  and  $S_3$  be known, then

$$k_1Q_1 = S_1 + k_3Q_3 + A_{12}k_2Q_2$$

$$k_2Q_2 = S_2 + A_{21}k_1Q_1$$

$$k_3Q_3 = S_3 + A_{31}k_1Q_1$$

and hence



$$Q_1 = \frac{S_1 + S_3 + A_{12}S_2}{k_1(1 - A_{31} - A_{12}A_{21})}$$

$$Q_2 = \frac{S_2 + A_{21}k_1Q_1}{k_2}$$

$$Q_3 = \frac{S_3 + A_{31}k_1Q_1}{k_3}$$

$$E_1 = A_{01}k_1Q_1$$

$$E_2 = A_{02}k_2Q_2$$

and

$$E_1 + E_2 = S_1 + S_2 + S_3.$$

Similarly, pool constants can be determined for other sets of constraints.

#### ANALYSIS OF MULTIPLE-POOL SYSTEMS BY MEANS OF PARTIAL SYSTEMS: AS ILLUSTRATED BY A HYPOTHETICAL $M$ METABOLIC SCHEME

As was shown<sup>(75)</sup>, the solutions of the tracer equations for a given  $n$ -pool system (i.e., for a given set of constants  $k_i$ 's and  $A_{ij}$ 's) can be found by means of Laplace Transforms<sup>(9, 18, 26, 69)</sup>. The actual approximation of the experimental data by a sum of more than two or three exponential functions, and subsequent evaluations of pool constants, may become unduly complicated, and even completely unfeasible, due both to the inadequacy and complexity of the available data.

Under certain circumstances, however, when the relative rates of transfer, as well as the roots of the characteristic polynomial, differ from each other sufficiently, such a system may be analyzed by separating it first into partial two- or three-pool systems. This procedure should not be confused with the lumping of several parts of the system into a single pool, or with the substitution of arbitrarily simplified solutions. Simplifications are valid only if they are based on an actual knowledge of the constants involved, or if they can approximate the exact solutions to a pre-assigned degree of accuracy.

Analysis by partial systems can be used only if such systems are accessible, and if there is no "significant" return to these systems during "sufficiently" long periods of time. The interpretation of the terms "significant" and "sufficient," as well as the accuracy of the derived constants, such as the relative rates of transfer, will depend to a large extent upon the errors inherent in the data. When the constants derived from the partial solutions are subsequently substituted into the general solutions, the resulting curves should approximate the experimental data within the limits determined by the experimental errors.

Let Figure 10 represent the model reflecting the kinetics of such a hypothetical five-pool system of  $M$  metabolism which is subject to the following conditions and interpretations:

$Q_1$  is the immediate miscible  $M$  pool in plasma. Part of the  $M$  leaving  $Q_1$  is excreted as  $E_1$ , the remainder transfers into  $Q_2$  and  $Q_5$ .

$Q_2$  is interstitial  $M$  pool which transfers only into pool  $Q_1$  so that  $A_{12} = 1$ .

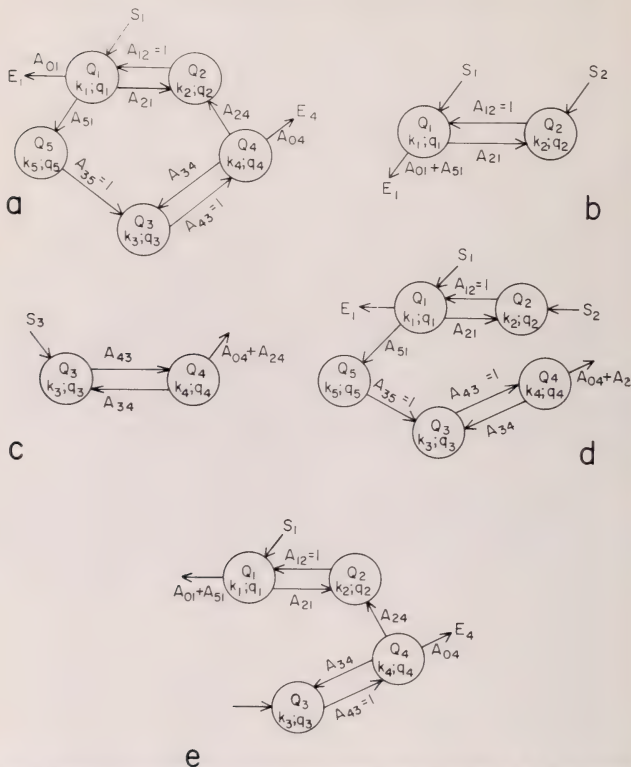


FIG. 10. Schematic representation of a hypothetical five-pool model of  $M - M_a$  metabolism (10a), and the partial sub-systems into which it may be separated to facilitate mathematical analysis (10b, c, d, e).

$Q_5$  is an  $M_a$  pool representing the product of anabolism of  $M$  within the organ of synthesis, which empties into  $Q_3$ , the  $M_a$  pool contained in plasma.  $Q_3$  in turn interchanges with  $Q_4$ , the remaining  $M_a$  pool comprising all the synthesized metabolite outside of  $Q_5$  and  $Q_3$ , so that  $A_{35} = A_{43} = 1$ . The  $M_a$  leaving  $Q_4$  is

partly returned to  $Q_3$ , partly excreted as  $E_4$  and partly is degraded and transferred to  $Q_2$  as  $M$ .

$S_1$  stands for the total intake of  $M$  which is exogenous to the system considered.

The analysis of this five-pool  $M - M_a$  model may then proceed by considering first the two partial two-pool systems consisting of  $Q_1Q_2$  (Fig. 10b) and  $Q_3Q_4$  (Fig. 10c) respectively. A simultaneous introduction of  $M$  and of  $M_a$  as two distinguishable radio- $M$  isotopes into the blood, i.e., into pools  $Q_1$  and  $Q_3$  respectively, permits the investigation of the two systems concurrently, without the risk of possible changes in the numerical values of the pool constants, which might take place during the time interval necessary between two successive administrations of a single isotope.

Data, such as the time of the appearance in one of the two-pool partial systems ( $Q_1Q_2$  or  $Q_3Q_4$ ) of detectable amounts of the isotope introduced into the other system, make it possible to determine the time intervals during which the partial systems represented in Figures 10b, c, d, and e, can be considered as independent of the rest of the system.

Once all experimental data have become available, and the time intervals for the validity of such data for each of the partial systems are determined, the actual mathematical analysis can be reduced to the following series of steps:

Step 1. Pool constants  $k_1$ ,  $k_2$  and  $A_{12}A_{21} = A_{21}$  are obtained from the approximating equation for radio- $M$  in  $Q_1$  of the partial system  $Q_1Q_2$  (Fig. 10b).

Step 2. Pool constant  $A_{01}$  (and therefore  $A_{51} = 1 - A_{21} - A_{01}$ ) is obtained from the total excretion from  $Q_1$  of radio- $M$  during the permissible initial interval  $t$ :

$$e_1(t)q_0 = A_{01} \int_0^t q_1(t) dt.$$

Step 3. Pool constants  $k_3$ ,  $k_4$  and  $A_{34}A_{43} = A_{34}$  are obtained from the approximating equation for radio- $M_a$  in  $Q_3$  of the partial system  $Q_3Q_4$  (Fig. 10c).

Step 4. Pool constant  $k_5$  can be obtained from the approximating equation for radio- $M$  in  $Q_3$  of the partial (non-cyclic) five-pool system  $Q_1Q_2Q_3Q_4Q_5$  (Fig. 10d), in conjunction with the previously determined constants. It should be noted that in practice this curve will consist of fewer than five exponential components, if within a short time period the component corresponding to  $e^{-\lambda_{1t}}$  of  $Q_1Q_2$  will have become completely negligible, and eventually further components may also vanish.

Step 5. Finally the pool constant  $A_{24}$  can be obtained from the excretion of catabolized radio- $M_a$  which now behaves as  $M$  and leaves the partial system  $Q_1Q_2Q_3Q_4$  (Fig. 10e) through  $Q_1$ .

Here again  $e^{-\lambda_{1t}}$  and  $e^{-\lambda_{3t}}$ , corresponding to the first exponential components of  $Q_1Q_2$  and  $Q_3Q_4$  respectively, may become negligible after a short time  $t$ .

Step 6. When the pool constants, determined by the above procedures, are substituted into the approximating equations for radio- $M$  or radio- $M_a$  corresponding to the complete five-pool model of Figure 10a, they should give a fair approxi-

mation to the experimental values extended over the period exceeding the maximum permissible time  $t$  for the partial systems. (On the other hand, the overall picture may become complicated by the phenomenon of recycling, i.e., passing of the isotopes through the organ of synthesis and subsequent time lag<sup>(53)</sup>.)

Step 7. Pool sizes: Assume for instance that the only pool available for quantitative measurement of stable isotope, is the plasma  $M_a$  pool  $Q_3$ . The amount of metabolite in the remaining pools ( $M$  in  $Q_1$  and  $Q_2$  and  $M_a$  in  $Q_4$  and  $Q_5$ ) can be determined either by use of Table IV, ref. 75, or by setting up equilibrium equations. Thus, from  $Q_3$  and the available pool constants we get:

$$\begin{aligned} Q_4 &= \frac{k_3}{k_4} Q_3 \\ Q_5 &= \frac{k_3}{k_5} Q_3 - \frac{A_{34}k_4}{k_5} Q_4 = \frac{k_3}{k_5} (1 - A_{34}) Q_3 \\ Q_1 &= \frac{k_5}{A_{51}k_1} Q_5 = \frac{k_3}{k_1} \frac{(1 - A_{34})}{A_{51}} Q_3 \\ Q_2 &= \frac{A_{21}k_1}{k_2} Q_1 + \frac{A_{24}k_4}{k_2} Q_4 = \frac{k_3}{k_2} \left[ \frac{A_{21}(1 - A_{34})}{A_{51}} + A_{24} \right] Q_3. \end{aligned}$$

This determines the system completely. Note that the number of conditions imposed upon the system of Figure 10a is exactly equal to the number of the degrees of freedom for a five-pool system under the assumptions of Table I, ref. 75, thus:

	Number of Con- di- tions
Conditions imposed upon $Q_1$ : $A_{11}k_1Q_1 = A_{31}k_1Q_1 = 0$	2
Conditions imposed upon $Q_2$ : $A_{42}k_2Q_2 = A_{32}k_2Q_2 = A_{52}k_2Q_2 = S_2 = E_2 = 0$	5
Conditions imposed upon $Q_3$ : $A_{23}k_3Q_3 = A_{13}k_3Q_3 = A_{53}k_3Q_3 = S_3 = E_3 = 0$	5
Conditions imposed upon $Q_4$ : $A_{14}k_4Q_4 = A_{34}k_4Q_4 = S_4 = 0$	3
Conditions imposed upon $Q_5$ : $A_{15}k_5Q_5 = A_{25}k_5Q_5 = A_{45}k_5Q_5 = S_5 = E_5 = 0$	5
Total number of imposed conditions:	20
Number of degrees of freedom for a five-pool system (see Table I, Ref. 75):	20

Hence any additional condition, such as  $E_4 = 0$ , for example, will no longer be independent of the equation constants and the preceding conditions.

#### GENERAL CONSIDERATIONS

In this paper we have constructed mathematical models for the first-order metabolic processes, as well as for more general processes, governed by the conditions of dynamic equilibrium. These models are subject to rigid assumptions which cannot be expected to hold in a complex living organism. The question therefore arises: Under what circumstances are these models applicable to living organisms, or more precisely, what minimal sets of mathematical assumptions

can replace physiological conditions? To some degree the stringency of such requirements is limited by the uncertainty inherent in the collection of experimental data. Experimental data available for mathematical analysis are subject to errors of sampling and sample preparation, as well as to statistical errors of counting. If changes, due to physiological variations in rates of transfer, pool sizes, etc., are random, and of the same order of magnitude as the experimental errors, they cannot in practice be distinguished from the latter. Under such conditions the rates of transfer may be considered constant. It is this type of situation that may be encountered in the analysis of plasma  $\text{Fe}^{59}$  disappearance data in the presence of variable iron concentrations in human plasma. The question of whether larger random variations may be acceptable will depend to some extent on the particular problems considered, and probably cannot be answered categorically.

As was mentioned before, the assumption of instantaneous mixing may be replaced by a less exacting requirement that the tracer leave each of the pools  $Q_i$  at the corresponding relative rate  $k_i$ . This means that  $k_i$  must be the same fraction both of the total substance  $Q_i$ , and of the tracer  $q_i$  leaving the  $i^{\text{th}}$  pool per unit time. Uniform mixing within a given pool is important only if the pool is used for sampling of the tracer, and if such sampling is random within the pool. The plasma iron pool during  $\text{Fe}^{59}$  disappearance determination illustrates the point. But even here the instantaneous mixing of the initial tracer dose  $q_0$  is not necessary as long as "uniform" mixing is achieved at the time of the first sampling, and the tracer is subject to the "constant" relative rate of transfer  $k_i$ . The word "constant" is to be understood within the context of the preceding paragraph. If sampling is not random the condition of complete mixing is no longer necessary. All that is required is that each sample be a fixed fraction of the total tracer present in the pool. As a concrete example we may consider the blood iodide pool where after a short initial period the iodide is distributed in a fixed ratio between plasma and red cell mass. Whether  $q_i$  and  $q_0$  refer to total pools in terms of microcuries, counts per second, or micrograms, or to some fixed fractions of the pools in terms of similar units, the final equations for  $\frac{q_i}{q_0}$  (i.e., for  $q_i$  normalized with respect to  $q_0$ ) will be the same.

In approximating empirical data by a sum of exponential functions, it is necessary to decide in advance upon the number of such functions. In general, this should be the lowest number of exponential functions yielding solutions within the limits of accepted experimental errors. If the exact form of some of the approximating functions has been predetermined by other experiments or considerations, the principle of the minimum number should be applied to the remaining functions. The resulting sets of "best" solutions are then unique in the sense that they differ from each other only within the confines of the above errors. Approximations by means of a larger number of functions, i.e., by more complicated systems, becomes meaningless insofar as such approximations are no longer unique or determinate, and as mere approximations might as well be represented by sets of entirely different functions. The danger of overcomplicating the ap-

proximating curves becomes very real when we consider the complexity of the human organism and then attempt to represent its metabolic processes by correspondingly complex mathematical systems. If one is not very careful, such attempts may deteriorate into a fruitless pursuit of mere curve fitting. On the other hand, such considerations do not justify oversimplifications. A simple model, even if it forms a good approximation to experimental data, does not necessarily reflect any part of the mechanism of the metabolic processes under study. A refinement of available data in such cases may sometimes lead to different and more useful models and predictions, as illustrated by the transition from a one-pool to a two-pool to a three-pool system in the case of plasma  $\text{Fe}^{59}$  disappearance studies.<sup>(51, 74)</sup>

In summary, a useful model should satisfy the following minimum requirements: 1. It should give a good approximation to experimental data. 2. It should conform in a general way to well established physiological principles. 3. It should give some additional information about the system under consideration, and should eventually be capable of an independent verification, pointing the way to further fruitful experimentation. It should be emphasized that even when a model fulfills all the above criteria, it remains merely a useful mathematical tool, enabling one to summarize in simple terms complex physiological phenomena.

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# Studies in Iron Kinetics:

## I. Interpretation of Ferrokinetic Data in Man

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The normal metabolism of iron is essentially endogenous. Only 0.5 to 1.5 mg of iron is absorbed and excreted per day as compared to 3 to 5 grams of total body iron content (1,2).

Plasma iron because of its ready accessibility and its transport function has assumed a central role in studies of this endogenous metabolism (3,4,5,6,7,8). Plasma iron is in an approximate state of dynamic equilibrium (with relatively large amounts of iron continuously leaving and entering), and quantitative chemical studies alone cannot yield adequate information about iron movement. Kinetic studies of radioiron in plasma have yielded considerable information about the dynamics of iron metabolism, especially in its relation to erythropoiesis (4,5,6,7,8). The red cell iron mass forms the largest iron compartment in the normal individual (1,2). Though it is as easily accessible as plasma, it cannot be directly labeled with radioiron. Studies of tracer behavior in mature red cells follow, therefore, the introduction of tracer iron via other routes (oral or parenteral means). When radioiron is injected into plasma, the rates of its removal from plasma and of its subsequent incorporation into the circulating erythrocytes can be measured (in vitro studies). The gross\* movement and distribution of the isotope over selected body sites may be followed by scanning technics (in vivo studies) (7,9,10,11). From such data models of iron metabolism have been formulated (4,7,8,12,13).

This paper presents ferrokinetic data analyzed in terms of various models of iron metabolism. The general assumptions underlying the formulations of such models are presented below. More detailed discussions of fundamental concepts as well as mathematical treatments with examples are given in other papers of this series (14,15,16,17).

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<sup>2</sup> Dazian Research Fellow.

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\* By "gross" is meant activity measured by the probe; relatively small changes may not be detectable.

## MATERIALS AND METHODS

*Subjects:* The experimental subjects were normal volunteers and patients hospitalized for various ailments. Healthy mongrel dogs of approximately 30 kg weight were used in the animal studies.

*Plasma  $Fe^{59}$  Disappearance and Red Cell  $Fe^{59}$  Uptake:* These procedures have been described in detail previously (4,11,19). Ten to twenty microcuries of  $Fe^{59}Cl_3$  of high specific activity (10 to 40 microcuries per microgram) were diluted with sodium citrate and incubated with beta-1-globulin (Cohn Fraction IV-7), with the subject's own plasma, or with autologous ascitic fluid. The latent iron binding capacity was five or more times that necessary to bind the total iron added. An aliquot containing 5 to 20 microcuries of  $Fe^{59}$  was injected intravenously (and in one patient, intraperitoneally), and blood samples were obtained at suitable intervals. Radioactivity was determined on samples of whole blood and plasma by counting in a well-type scintillation counter with a thallium-activated NaI crystal, either for 25,600 counts or for a minimum of 100 minutes. Plasma volumes were determined from the plasma  $Fe^{59}$  disappearance data by extrapolation to zero time and estimation of the tracer dilution.

$Fe^{59}$  activity in red cells was measured during the subsequent two to three weeks and the percent incorporation estimated from whole blood  $Fe^{59}$  activity and red cell mass determined by  $Cr^{51}$  (20).

*Body Surface Counting Rates (10,11,16):* With the subject supine, scintillation crystal counters, suitably shielded and collimated, were directed at marrow (posterior sacral region), liver and splenic sites (right and left anterior axillary lines respectively, at the level of the 9th and 10th ribs or subcostally). The gross counting rates at any given time were corrected for the radioactivity in the circulating blood.\* The net radioactivity over each site was extrapolated to zero time,  $t_0$ . This represented the initial radioactivity due to circulating blood (plasma) only. Similarly, radioactivity per 2 ml whole blood was extrapolated to zero time. Let  $SCR(t)$  = the surface counting rate;  $WBCR(t)$  = the whole blood counting rate (per 2 ml); and  $CSCR(t)$  = the corrected surface counting rate at time  $t$ .

$$\text{Then } CSCR(t) = SCR(t) - WBCR(t) \frac{SCR(t_0)}{WBCR(t_0)}.$$

*Determination of Lymph  $Fe^{59}$  Activity:*

1. Dogs: Under light nembutal anesthesia the thoracic duct was catheterized using either a transcervical or transthoracic approach (21). The lymph was not recirculated, and intravenous infusions were administered for hydration.  $Fe^{59}$  tracer was injected intravenously immediately after catheterization, and lymph and blood were serially sampled. The lymph flow was 0.25 to 0.5 ml per minute; the mid-point of each collection interval was used as the sample time.

\* Both corrected and uncorrected data are used in *in vivo* studies (6,7,9,10,11,18). In the present studies where the interest centers in multiple-pool analysis, the *in vivo* data are decomposed into various relevant components. This can be accomplished only after a preliminary correction for circulating blood background.

2. Patients: The thoracic duct, catheterized under local anesthesia via a transcervical approach (22,23), produced a lymph flow rate of 0.5 to 1 ml per minute. The  $\text{Fe}^{59}$  studies were performed one to three days after surgery.

Both in dogs and in patients the catheter dead space was negligible in relation to the rate of lymph flow, and no correction for this was deemed necessary.

*Iron Concentration and Iron Binding Capacity:* The concentration of iron in the plasma and lymph was determined by the method of Ramsey (24) or of Schade (25) and the iron binding capacity by that of Ventura (26) or of Schade (25).

*Electrophoresis and Radioactive Strip Scanning:* Paper electrophoresis was performed on samples of plasma, lymph and transudates, and the  $\text{Fe}^{59}$  radioactivity was localized by means of an automatic strip scanner with a thin window methane gas flow counter.\* Acrylamide gel electrophoresis was performed according to the method of Ornstein and Davis (27).

*Theoretical and Mathematical Considerations:* (For definitions and proofs see references (8,13,14,15,16,17)).

The experimental radioiron data have been evaluated in terms of multiple-pool models of the initial "preerythropoietic phase" of iron kinetics. This "pre-erythropoietic phase" refers to interchange of the metabolite among the several compartments through which intravenously injected radioiron is distributed prior to its irreversible fixation in the maturing erythron. The formulation of these models was motivated by the fact that the majority of the pertinent data, such as plasma radioiron disappearance, red cell radioiron uptake, and regional localization of the tracer as determined by body surface scanning can be approximated by linear combinations of a few (usually three) exponential functions with constant coefficients (8,14,16,18).

$$q_i = \alpha_{i1}e^{-\lambda_1 t} + \alpha_{i2}e^{-\lambda_2 t} + \alpha_{i3}e^{-\lambda_3 t} + \cdots + \alpha_{in}e^{-\lambda_n t}.$$

(When there is no ambiguity  $\alpha_{i1}$  can be replaced by  $\alpha_1$ ,  $\alpha_{i2}$  by  $\alpha_2$ , etc.). Such linear combinations of exponential functions are solutions of first order homogeneous linear differential equations with constant coefficients,

$$\frac{dq_i}{dt} = -k_i q_i + \sum_{\substack{j=1 \\ j \neq i}}^n A_{ij} k_j q_j \quad \text{for } i = 1, 2, \cdots n$$

and can be interpreted as describing radioiron (as well as stable isotope) movement between several distinct compartments of iron metabolism. (In the above,  $q_i$ 's and  $t$  are the dependent and independent variables respectively while  $k_i$ 's and  $A_{ij}$ 's are constants. (See captions Figs. 1 and 2). Such mathematical models cannot refer directly to biological phenomena; they should, however, provide theoretical counterparts to the phenomena which they are supposed to explain. In the case of metabolic studies, we assume that the underlying physiological

\* These procedures were kindly performed by Dr. Milton Mendlowitz, Dr. Robert L. Wolf and Mrs. Julia Roboz.



processes are approximately subject to the following set of definitions and assumptions:

Distinct compartments or pools are aggregates of the basic substance which differ from each other spatially or in the physiological properties of the metabolite, in this particular case, iron. The total rate with which radioiron (or stable isotope) leaves an individual compartment is always proportional to the radioactivity,  $q$ , (or the amount  $Q$  of the stable isotope) in that compartment. The fractional amount of total radioactivity (or stable isotope) leaving a given pool, which enters another specified pool, or leaves the system as a whole, is always constant. This implies that either all transfer rates are equivalent to first order reactions, or that the metabolic system is in dynamic equilibrium, i.e., equal amounts of the stable isotope enter and leave a given individual compartment or a given subset of pools as a whole. This in turn implies that the mixing of radioisotope with the stable substance is almost instantaneous, both during the initial injection of the tracer and during subsequent continuous interchanges or transfers of the metabolite containing varying concentrations of the radioisotope. The terms "transfer rates" and "interchanges" refer either to spatial transfers and interchanges, or to physiological changes in the metabolite. In general, when the same "accessible" (8) pool is used for an instantaneous introduction of a single tracer dose, and as a sampling pool,  $n$  exponential functions of the approximating tracer equation indicate the existence of at least  $n$  interchanging pools (8,14) of a "reduced" pool system, i.e., a system where some of the metabolite (and therefore isotope) moves from each pool to every other pool, possibly via some intermediary pools. Such a reduced three-pool system is depicted in Figures 1 and 2.

Solutions of the "reduced"  $n$ -pool systems describing the tracer behavior in various compartments, consist, as a rule, of linear combinations of  $n$  exponential components. The set of the  $n$  exponents ( $-\lambda_i t$ 's) is the same for each of the  $n$  compartments, while the coefficients ( $\alpha_{ij}$ 's) vary both with the particular pool ( $Q_i$ ) considered and with the "accessible" compartment used for the introduction of the initial tracer dose  $q_{oi}$ . When the pool constants (i.e.,  $k_i$ 's and  $A_{ij}$ 's) are known, these solutions can be derived from the given differential equations by means of Laplace transforms (14,28,29,30). In practice, however, the situation is reversed and the pool constants must be determined from the known tracer behavior in one or in several compartments of the system, or rather from the best approximations to such behavior. In this context a "best approximation" to experimental data implies an approximation within the limits of experimental errors by a sum of a minimum number of exponential functions  $\alpha_{ij}e^{-\lambda_j t}$ 's. Once the pool constants are known, the tracer behavior in all pools can be determined (14,16,17,31).

Plasma is at present the only well delineated accessible iron pool, and, as such, it forms a logical starting point for most iron kinetic studies. Its ready accessibility permits an "instantaneous" introduction of an initial physiological tracer dose, as well as adequate subsequent sampling, without interfering with, or causing any detectable changes in the steady state of the metabolite.

The first attempts at mathematical analysis of plasma  $\text{Fe}^{59}$  disappearance data resulted in the approximation of the experimental data during the first two hours after an intravenous injection of  $\text{Fe}^{59}$  by a single exponential function

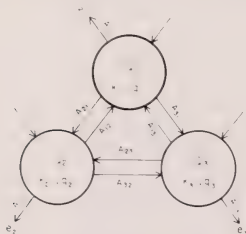


FIG. 1.

FIG. 1. Schematic representation of a general reduced three-pool system.  $Q_i$  designates both the  $i^{\text{th}}$  pool and the amount of metabolite in the  $i^{\text{th}}$  pool.  $q_i$  represents the amount of radioisotope in the  $i^{\text{th}}$  pool.  $k_i$  is the relative rate; i.e., the fraction of the total metabolite (or of the radioisotope) in the  $i^{\text{th}}$  pool which leaves the  $i^{\text{th}}$  pool per unit time.

$A_{ij}$  is the interchange constant which is defined as that fraction of the total substance leaving the  $i^{\text{th}}$  pool, which leaves the  $i^{\text{th}}$  pool for the  $j^{\text{th}}$  pool. In particular  $A_{ii}$  is such fraction leaving the system as a whole.

$e_i$  represents the fraction of the initial radioactivity,  $q_{0i}$ , which eventually leaves the system via the pool  $Q_i$ .

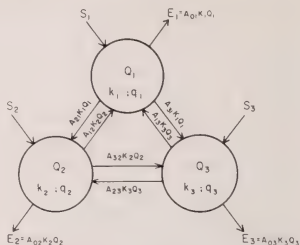


FIG. 2.

FIG. 2. Schematic representation of a general reduced three-pool system. Here the actual amounts of the metabolite transferred from one pool to another, or leaving the system as a whole, are indicated by annotated arrows (For the exact meaning of the notation see legend of Fig. 1.). For instance  $A_{21}k_1Q_1$  is the amount of metabolite transferred from the pool  $Q_1$  to pool  $Q_2$  per unit time.  $S_i$  and  $E_i$  are the amounts of the metabolite which enter and leave the system as a whole per unit time through the  $i^{\text{th}}$  pool  $Q_i$ .

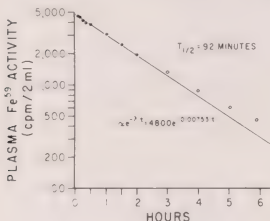


FIG. 3. Subject S.H. (Cerebral Thrombosis): Approximation of plasma  $\text{Fe}^{59}$  disappearance data during the first two hours of the experiment by a single exponential function. ●●● Experimental data during the first two hours of the experiment approximated by the equation

$$q = \alpha e^{-\lambda t} = 4800e^{-0.00753t}$$

where  $t$  is time in minutes. ○○○ Experimental data after the first two hours of the experiment. (For exact values of plasma  $\text{Fe}^{59}$  disappearance data see Table I.)

(Fig. 3 and Table I):

$$q = \alpha e^{-\lambda t}.$$

This single pool model of Huff (Fig. 4 and Table I) (4) is the only model whose tracer distribution system (consisting of pool constants which are independent of the amounts of the metabolite, such as relative rates  $k_i$  and interchange and exit constants  $A_{ij}$  and  $A_{oi}$ ) is always completely determined by the approximating



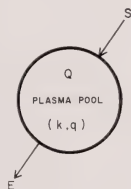
equation. If, in addition,  $Q_1$ , the amount of the metabolite in plasma is also known, the steady state system is determined as well ( $S = E = kQ = \text{Iron Turnover}$ ).

TABLE I

Subject S.H. (Cerebral thrombosis). Approximation of experimental plasma  $\text{Fe}^{59}$  disappearance data by one (I), two (II) and three (III) exponential functions, respectively

Time after $\text{Fe}^{59}$ injection (min)	Activity in plasma in cpm/2 ml	I		II		III			
		A $4800e^{-0.0753t}$	B $4050e^{-0.00356t}$	C $700e^{-0.00231t}$	D B + C	E $4200e^{-0.00063t}$	F $460e^{-0.00233t}$	G $100e^{-0.00329t}$	H E + F + G
5	4610	4620	3880	690	4570	4022	455	100	4575
10	4450	4450	3718	681	4400	3851	449	100	4400
15	4170	4285	3562	671	4235	3688	444	100	4230
20.5	3950	4115	3398	660	4060	3517	438	99	4055
30	3810	3830	3133	643	3775	3237	429	99	3765
62	3100	3010	2382	587	2970	2455	397	98	2950
91	2480	2420	1859	541	2400	1909	371	97	2375
120	1960	1945	1450	498	1950	1485	347	96	1930
180	1310	1235	868	420	1290	883	301	94	1280
240	875	788	519	354	875	525	262	92	880
302	605	494	305	297	600	307.0	226.2	90.5	625
354	459	334	196	256	452	195.7	200.2	89.0	485
720	177.2	21.1	4.1	90.6	94.7	8.4	84.7	78.9	172.0
1380	78.6			13.9	13.9		9.9	62.2	72.0
2880	38.9			.3				38.7	38.7
4320	24.8							24.1	24.1

FIG. 4. Schematic representation of a single-pool system consisting of the plasma iron pool. Here  $Q$  and  $q$  denote the amounts of stable and radioactive iron respectively.  $E = S = Qk$  is the amount of iron leaving plasma per unit time.



When the period of experimentation is extended to about six hours, two exponential functions,

$$q_1 = \alpha_1 e^{-\lambda_1 t} + \alpha_2 e^{-\lambda_2 t},$$

become necessary to achieve an adequate approximation to plasma radioiron data (Fig. 5 and Table I) (12), indicating the existence of at least two initial mutually interchanging pools (Fig. 6). This system is indeterminate and has two

degrees of freedom (14), when only the constants of the approximating equation to plasma  $\text{Fe}^{59}$  disappearance data, (and the value  $Q_1$  in the case of the steady state system) are known. (This model-indeterminacy is indicated by broken arrows in Fig. 6.) In order to obtain complete mathematical solutions, two

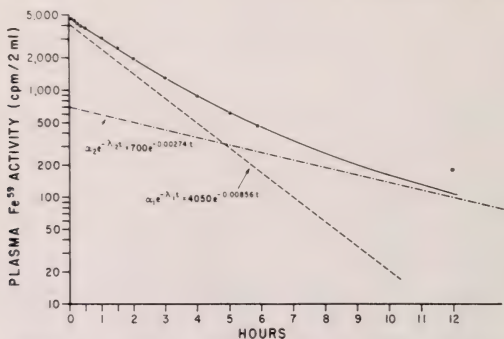


FIG. 5. Subject S.H. (Cerebral Thrombosis): Approximation of plasma  $\text{Fe}^{59}$  disappearance data during the first six hours of the experiment by a sum of two exponential functions.  $\bullet \bullet \bullet$  Experimental data during the first six hours of the experiment, approximated by the equation

$$q_1 = \alpha_1 e^{-\lambda_1 t} + \alpha_2 e^{-\lambda_2 t} = 4050 e^{-0.00856 t} + 700 e^{-0.00274 t}$$

○ Experimental value at twelve hours.

--- Exponential function  $\alpha_1 e^{-\lambda_1 t} = 4050 e^{-0.00856 t}$

- · - · - Exponential function  $\alpha_2 e^{-\lambda_2 t} = 700 e^{-0.00274 t}$

(For exact values of plasma  $\text{Fe}^{59}$  disappearance data see Table I.)

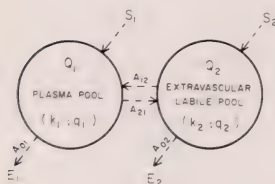


FIG. 6. Schematic representation of a two-pool system consisting of a plasma iron pool and a second pool (extravascular labile iron pool).

constraints must be imposed upon the system, which can be achieved in terms of assumptions about the modes of exit and entry of the metabolite (8,12,14).

Currently, it has been demonstrated (7,8,17,32,33) that experimental plasma  $\text{Fe}^{59}$  disappearance data during the first 48 hours (and sometimes for as long as ten to twenty days) can best be approximated by a sum of three exponential functions (Fig. 7 and Table I):

$$q_1 = \alpha_1 e^{-\lambda_1 t} + \alpha_2 e^{-\lambda_2 t} + \alpha_3 e^{-\lambda_3 t}$$

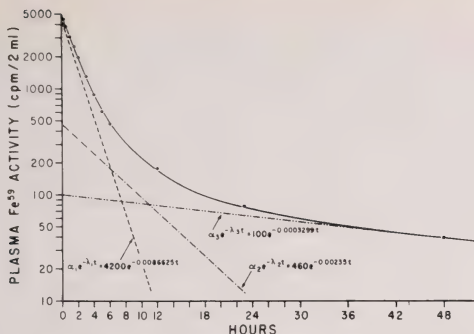


FIG. 7. Subject S.H. (Cerebral Thrombosis): Approximation of plasma  $\text{Fe}^{59}$  disappearance data during the first two days of the experiment by a sum of three exponential functions. ••• Experimental data during the first ten days of the experiment, approximated by the equation:

$$q_1 = \alpha_1 e^{-\lambda_1 t} + \alpha_2 e^{-\lambda_2 t} + \alpha_3 e^{-\lambda_3 t} = 4200e^{-0.008665t} + 460e^{-0.00235t} + 100e^{-0.0003299t}$$

where  $t$  is time in minutes.

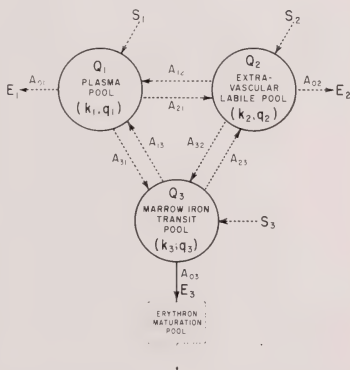
--- Exponential function  $\alpha_1 e^{-\lambda_1 t} = 4200e^{-0.008665t}$

----- Exponential function  $\alpha_2 e^{-\lambda_2 t} = 460e^{-0.00235t}$

..... Exponential function  $\alpha_3 e^{-\lambda_3 t} = 100e^{-0.0003299t}$

(For exact values of plasma  $\text{Fe}^{59}$  disappearance data, see Table I.)

FIG. 8. Schematic representation of the general "reduced" three-pool system of the "pre-erythropoietic phase" of iron metabolism, consisting of plasma, and two other pools to be identified as extravascular labile, and marrow iron transit pools. The fact that the exact interconnections of the three pools and the modes of exit and entry of the metabolite are not known, is indicated by the broken arrows. In this figure the reduced three-pool system is connected unidirectionally with the erythron maturation pool. For the exact notation and interpretation, see text and Figs. 1 and 2.



This implies the existence of at least three distinct mutually interchanging compartments (14) of the initial "preerythropoietic phase" of iron metabolism (Fig. 8) (see below). Here again the exact interconnections of the component pools of the reduced three-pool system cannot be derived from the plasma  $\text{Fe}^{59}$

disappearance data, and the amount of stable iron in plasma, alone. This system has six degrees of freedom (14) and requires six constraints for a unique solution. The six constraints can be imposed in terms of two interchange constants and four constants of the modes of exit and entry.

From a purely mathematical point of view, the constant parts of the three exponents of the approximating equation ( $-\lambda_1$ ,  $-\lambda_2$  and  $-\lambda_3$ ) represent simply the three real roots of a third degree equation whose coefficients are formed by combinations of pool constants ( $k_i$ 's and  $A_{ij}$ 's). Linear combinations of the corresponding exponential functions,  $\alpha_{ij}e^{-\lambda_j}t$ 's (where  $\alpha_{ij}$ 's are in turn expressions in terms of the same pool constants), describe the tracer behavior in each of the

TABLE II

*Subject J.O. (Normal). In vivo radioactivity data over various body sites during the first six hours of the experiment*

Time after inj. (min.)	Bone Marrow Area		Spleen Area		Liver Area	
	Gross counts per unit time	Counts corrected for blood background	Gross counts per unit time	Counts corrected for blood background	Gross counts per unit time	Counts corrected for blood background
5	19.3	1.6	61.5	1.9	75.5	2.7
10	19.9	2.5	59.4	1.1	74.6	3.4
18	21.5	5.5	58.2	4.3	73.4	7.7
30	22.3	7.4	56.7	6.8	73.5	12.5
45	24.3	11.3	55.4	11.6	73.5	20.0
60	25.7	13.5	54.9	14.0	73.2	23.3
90	29.0	18.9	53.7	19.6	74.2	32.6
120	31.0	22.6	53.4	23.2	71.7	36.2
150	32.5	25.4	51.6	27.9	72.2	43.3
240	34.8	30.9	51.1	37.9	72.0	55.9
355	38.9	36.1	49.6	40.3	72.6	61.3

three respective pools. (None of these exponential functions is, by itself, characteristic of any one of the several component pools of the system.)

## RESULTS

I. Body Surface Counting: In attempting to identify the "second pool" most rapidly interchanging iron with plasma (the first pool) during the initial four to eight hours, it seemed reasonable to assume an "extravascular labile pool" in the interstitial fluid. It was postulated that the existence of such a "second pool" may be reflected in body surface counting. Typical body surface counting rate data,\* over liver, spleen and bone marrow, in two subjects, one normal and one with anerythropoietic anemia, are represented in Figs. 9 and 10, and Tables II and III. Similar in vivo results were obtained over multiple sites (including marrow, liver, spleen and soft tissue such as mid-thigh and calf) in more than 65 other patients.

\* The body surface radioactivity consists of radiation from all tissues "seen" directly by the probe as well as scattered radiation from surrounding sites (16).

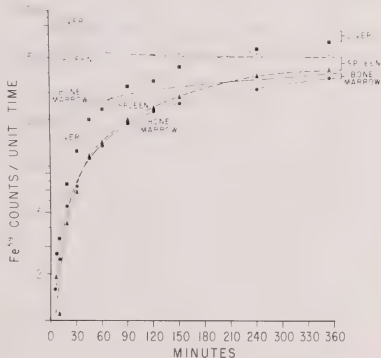
Corrected experimental body surface  $\text{Fe}^{59}$  activity data for the first six hours of the study could be decomposed into 1) a component representing  $\text{Fe}^{59}$  in combined erythropoiesis and storage, and 2) a component representing  $\text{Fe}^{59}$  in another pool, rapidly interchanging with plasma. Such decompositions were

TABLE III

*Subject M.M. (Anerythropoietic Anemia). In vivo radioactivity data over various body sites during the first five hours of the experiment*

Time after inj. (min)	Bone Marrow Area		Spleen Area		Liver Area	
	Gross counts per unit time	Counts corrected for blood background	Gross counts per unit time	Counts corrected for blood background	Gross counts per unit time	Counts corrected for blood background
7.5	26.3	1.0	74.0	2.1	85.6	2.3
15	26.5	1.6	74.8	4.2	85.3	3.5
30	26.9	3.3	73.2	6.4	90.1	12.7
46	26.7	3.9	74.2	9.4	92.4	17.2
63	28.2	6.0	75.2	12.3	100.8	28.0
90	28.5	7.6	75.0	15.6	104.5	35.7
120	29.4	10.3	79.4	25.3	108.7	46.0
153	30.8	13.0	72.2	21.7	106.3	47.8
214	30.3	14.9	71.9	28.1	109.5	58.8
300	32.2	20.1	71.7	37.4	123.3	83.6

FIG. 9. Subject J.O. (Normal): In vivo radioactivity over various body sites during the first six hours of the experiment.  $\circ-\circ-\circ$  Gross  $\text{Fe}^{59}$  activity over bone marrow area.  $\dots$   $\text{Fe}^{59}$  activity over bone marrow area, corrected for blood background.  $\triangle-\triangle-\triangle$  Gross  $\text{Fe}^{59}$  activity over spleen area.  $\blacktriangle-\blacktriangle-\blacktriangle$   $\text{Fe}^{59}$  activity over spleen area, corrected for blood background.  $\square-\square-\square$  Gross  $\text{Fe}^{59}$  activity over liver area.  $\blacksquare-\blacksquare-\blacksquare$   $\text{Fe}^{59}$  activity over liver area, corrected for blood background.



performed on data obtained over various body sites in thirty subjects, some normal and some with hematologic disorders (16). In Fig. 11, which is typical of such analysis, the data over the splenic region are decomposed for the mode of exit of iron through either the first (plasma) or the second (extravascular labile) pool respectively (model groups I and II) (14).

It has been shown (16) that any set of data which can be decomposed into the

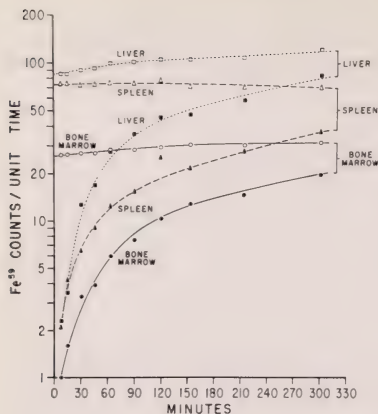


FIG. 10. Subject M.M. (Anerythropoietic anemia): In vivo radioactivity over various body sites during the first five hours of the experiment.  $\circ-\circ-\circ$  Gross  $\text{Fe}^{59}$  activity over bone marrow area.  $\bullet-\bullet-\bullet$   $\text{Fe}^{59}$  activity over bone marrow area, corrected for blood background.  $\triangle-\triangle-\triangle$  Gross  $\text{Fe}^{59}$  activity over spleen area.  $\blacktriangle-\blacktriangle-\blacktriangle$   $\text{Fe}^{59}$  activity over spleen area, corrected for blood background.  $\square-\square-\square$  Gross  $\text{Fe}^{59}$  activity over liver area.  $\blacksquare-\blacksquare-\blacksquare$   $\text{Fe}^{59}$  activity over liver area, corrected for blood background.

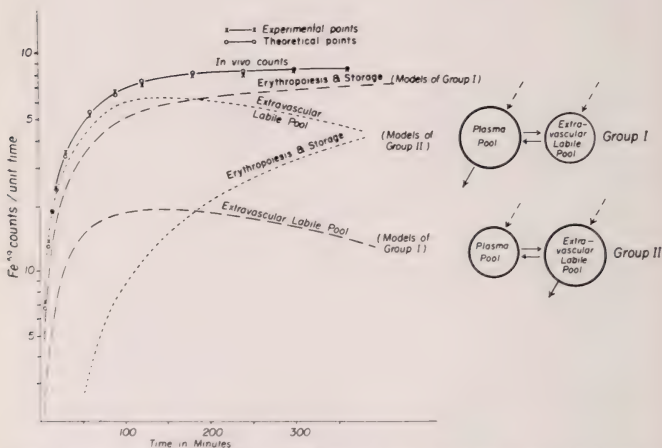


FIG. 11. Analysis of typical in vivo  $\text{Fe}^{59}$  activity over splenic area based on the assumption of two different groups of models (group I and group II respectively). The curve of net body surface radioactivity (corrected for the blood background) over the splenic area has been decomposed into the activity derived from the extravascular labile pool, and that from the combined erythropoietic and storage sites, during the first six hours of the experiment.

above two components for a given two-pool model, can be decomposed similarly into the corresponding components for any other two-pool system. The differences between empirical and theoretical values were in all cases within the limits of experimental errors.

It should be noted that the theoretical tracer behavior values in the second pool as calculated on the basis of an initial three-pool system, do not differ significantly from approximations based upon an initial two-pool system, during the first four to six hours of observation (i.e., as long as the two approximations to the experimental plasma data are equally satisfactory).

As has been shown, *in vivo* curves over all observed sites can be decomposed into two components, one of which follows the predicted (i.e., calculated from plasma  $\text{Fe}^{59}$  disappearance data on the basis of a two-pool system) tracer behavior in the second iron pool. This gives strong, although indirect, support to the hypothesis that the second iron pool rapidly interchanging with plasma during the first few hours of the experiment is iron in the interstitial fluid, the "extravascular labile pool."

The initial phase of *in vivo*  $\text{Fe}^{59}$  behavior has been described above. Results of ferrokinetic studies on a patient, F.A., including the later phase of *in vivo*  $\text{Fe}^{59}$  behavior (days 1 through 15) are shown in Fig. 12. The plasma iron disappearance data for 11 days are approximated by a sum of three exponential functions. The radioactivity for the third and successive days over the spleen and liver sites has been decomposed into a storage component and an exponential component representing the rate of removal of  $\text{Fe}^{59}$  from scanned erythropoietic areas. Over the bone marrow site, only the exponential component was detected. This exponential component has almost the same slope for the spleen, liver and bone marrow, and is seen again as the third component of the approximating curve to the plasma  $\text{Fe}^{59}$  disappearance data. It is also reflected, with a negative coefficient, in the red cell uptake data. Similar results were obtained on 25 other subjects. This consistent appearance of the same exponential function indicates the interrelationship between the "preerythropoietic" and "erythropoietic" phases of iron metabolism.

II. Observations on Pleural, Ascitic and Edema Fluids: Observed values for iron concentration and iron binding capacity in specimens of pleural, ascitic and edema fluids are given in Table IV. All samples of effusion fluids and most samples of edema fluid contained 10% or more of the iron concentration and total iron binding capacity of the corresponding plasma.

Paper electrophoresis and acrylamide gel electrophoresis of all fluids tested exhibited a band with the mobility of siderophilin and with the radioactive iron localized to this band.

The interchange of radioiron between plasma and transudates was studied in patients with ascites, pleural effusion and generalized anasarca. Following the intravenous injections of protein bound  $\text{Fe}^{59}$ , the transfer of radioiron to these fluids was observed (Tables V, VI, VII and Figs. 13, 14, 15). Conversely, the intraperitoneal injection of ascitic fluid bound  $\text{Fe}^{59}$  in a patient with ascites, showed a transfer of radioiron to plasma (Table VIII, Fig. 16). These studies



demonstrate interchange of iron between plasma and abnormal interstitial fluids.

III. Observations on Lymph: Values for the concentration of iron, and iron binding capacity in thoracic duct lymph in dogs and human subjects are given in Table IX. The concentrations were of the same order of magnitude in the central lymph as in the corresponding plasma. Electrophoresis of lymph demon-

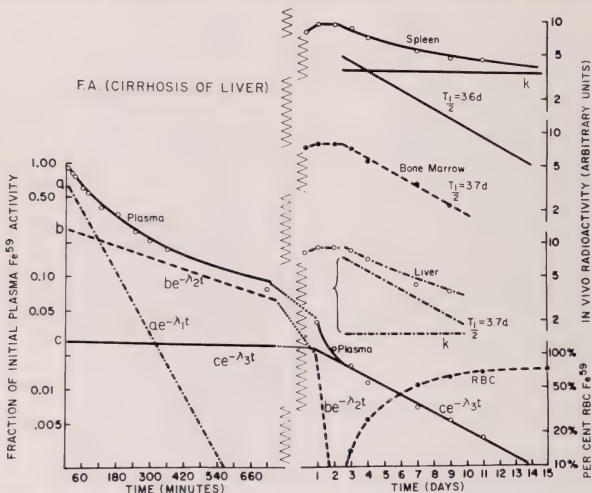


FIG. 12. Subject F.A. (Cirrhosis of Liver): Experimental  $\text{Fe}^{59}$  plasma disappearance, red cell uptake and surface radioactivity data.  $\circ\circ\circ$  Plasma  $\text{Fe}^{59}$  disappearance data.  $\bullet\bullet\bullet$   $\text{Fe}^{59}$  red cell uptake data. The plasma  $\text{Fe}^{59}$  data are shown decomposed into a sum of three exponential functions. The body surface radioactivity over the liver and spleen sites are decomposed into a constant shown as a horizontal line,  $k$ , and into an exponential component. Over the marrow only an exponential component is present.

strated a band with the mobility of plasma siderophilin, and radioactive strip scanning showed localization of radioactivity only to the same band.

Simultaneous observations of plasma and of lymph  $\text{Fe}^{59}$  tracer behavior, were performed on nine dogs and four patients after intravenous injection of radio-iron. The experimental findings in two dogs are noted in Tables X and XI, and Figs. 17 and 18. Lymph radioactivity increases and achieves a maximum at approximately 90 and 60 minutes, respectively. After about three hours the lymph radioactivity begins to parallel plasma  $\text{Fe}^{59}$  disappearance when plotted on semilogarithmic paper. The experimental plasma radioactivity data are approximated by a sum of two exponential functions (Tables XII, XIII) assuming an initial two-pool system. This approximation is used for the calculations of

TABLE IV

*Comparative concentrations of iron and total iron binding capacity (in  $\mu\text{g}$  per 100 ml) in plasma and in pathological accumulations of interstitial fluids*

Patient	Plasma		Ascitic Fluid		Pleural Fluid		Edema Fluid	
	Fe	TIBC	Fe	TIBC	Fe	TIBC	Fe	TIBC
A.A.	60	244			26	70	16	30
M.A.	83	217					3	7
S.B.	120	183					13	33
A.C.	73	343	38	180				
C.F.	40	262			28	133		
A.H.	40	352			30	139		
E.H.	37	245					8	8
Hy.	44	61					0	0
E.L.	53	356	30	147				
R.L.	98	397			40	313		
T.L.	65	399	98					
J.M.	124		84	159				
E.P.	35	177					45	
T.S.	38	240	85				45*	45*
							15†	15†
We.	60	188					50	103

\* left leg.

† right leg.

TABLE V

*Subject E.P. (Nephritis). Experimental plasma  $\text{Fe}^{59}$  disappearance and edema fluid  $\text{Fe}^{59}$  appearance data, following an intravenous injection of  $\text{Fe}^{59}$  beta-1-globulin*

Time after $\text{Fe}^{59}$ injection (min)	Activity in plasma cpm, 2 ml	Activity in edema fluid cpm, 2 ml
5	4865	
10	4615	
20	3990	
30	3660	
63	2420	
76		13.2
86	1810	
150	955	
188		8.0
210	595	
223		2.8
270	475	
308		2.8

the theoretical tracer behavior in the second, extravascular labile, pool (Tables XII, XIII).

The observed tracer behavior in plasma and in thoracic duct lymph of a patient with mild iron deficiency anemia is given in Table XIV and Fig. 19. The plasma

radioactivity data are approximated by a sum of two and of three exponential functions (Table XV) assuming initial two- and three-pool models respectively. These approximations are used for calculations of the theoretical tracer behavior

TABLE VI

*Subject A.L. (Nephritis). Experimental plasma  $Fe^{59}$  disappearance and ascitic fluid  $Fe^{59}$  appearance data following intravenous injection of  $Fe^{59}$  beta-1-globulin*

Time after $Fe^{59}$ injection (min)	Activity in plasma cpm/2 ml	Activity in ascitic fluid cpm/2 ml
5	3615	0
8		
12	3190	
20	3110	
30	2200	
31		8.6
62	1340	
63		12.6
110	703	
112		19.5
180	320	
225		24.4
248	214	
255		25.8
300	162.5	
312		23.8
347	134.2	

TABLE VII

*Subject A.A. (Heart Failure). Experimental plasma  $Fe^{59}$  disappearance and pleural fluid and edema fluid  $Fe^{59}$  appearance data after intravenous injection of  $Fe^{59}$  tagged autologous plasma*

Time after $Fe^{59}$ injection (min)	Plasma $Fe^{59}$ in cpm/2 ml	Pleural Fluid $Fe^{59}$ in cpm/2 ml	Edema Fluid $Fe^{59}$ in cpm/2 ml
10		1.10	1.51
12	12,800		
20	11,100	2.28	
30	8,890	2.01	17.4
45		5.58	11.1
47	7,140		
60	5,840	7.52	
75	4,350		
90	3,620	16.6	
18 hours	30.7	63.6	54.5

in the second, extravascular labile, pool (Table XVI). Similar calculations based on an initial two-pool model (Table XVII) were performed on the experimental data (Table XVIII and Fig. 20) of a patient with cirrhosis of the liver.

Experimental results on two additional patients and on three dogs are given

in Tables XIX to XXIII and Figs. 21 to 24. The tracer behavior patterns are similar to those described above.

#### IV. Quantitation of Results: The experimentally obtained $\text{Fe}^{59}$ plasma dis-

TABLE VIII

*Subject A.C. (Kimmelstiel-Wilson Disease): Experimental plasma  $\text{Fe}^{59}$  appearance and ascitic fluid  $\text{Fe}^{59}$  disappearance data following intraperitoneal injection of  $\text{Fe}^{59}$  bound to autologous ascitic fluid*

Time after $\text{Fe}^{59}$ injection into peritoneal cavity (min)	Activity in ascitic fluid cpm/2 ml	Activity in plasma cpm/2 ml
12.5		2.08
15	8150	
30		3.29
32	7445	
44		9.34
52	6975	
60	6955	
62		14.15
91		27.4
92	6555	
120		53.2
121	6225	
152		64.2
154	5900	
180	5665	
183		80.4
211	5520	
213		86.6

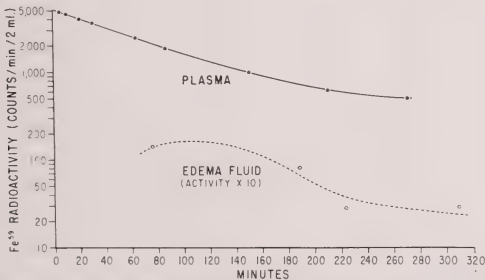


FIG. 13. Subject E.P. (Nephritis): Radioactivity in plasma and edema fluid following an intravenous injection of  $\text{Fe}^{59}$ -beta-1-globulin. •---• Experimental plasma  $\text{Fe}^{59}$  data. ○---○---○ Experimental edema fluid  $\text{Fe}^{59}$  data (multiplied by 10).

appearance and red cell uptake data have been analyzed on the basis of one-, two- and three-pool models. The size and turnovers of the component iron pools in four normal volunteers and patient S.H. are given in Table XXIV for the

four three-pool models depicted in Fig. 25. Fig. 26 illustrates these results for models IIIa and IIIb for S.H. (See discussion and Figs. 29, 30).

Tables XXV and XXVI contain similar quantitative data on twenty subjects

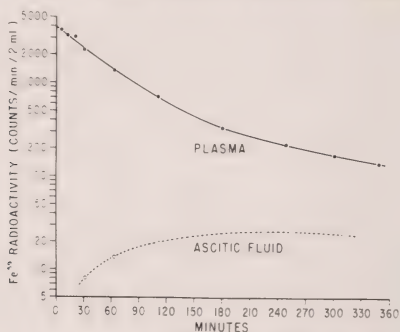


FIG. 14. Subject A.L. (Nephritis): Radioactivity in plasma and ascitic fluid following an intravenous injection of  $\text{Fe}^{59}$ -beta-1-globulin. ••• Experimental plasma  $\text{Fe}^{59}$  data. ○-○-○ Experimental ascitic fluid  $\text{Fe}^{59}$  data.

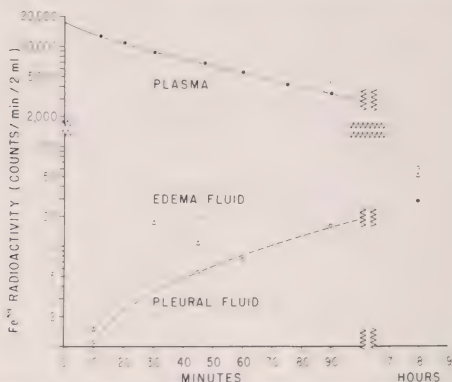


FIG. 15. Subject A.A. (Heart Failure): Experimental plasma  $\text{Fe}^{59}$  disappearance and pleural fluid and edema fluid  $\text{Fe}^{59}$  appearance data after an intravenous injection of  $\text{Fe}^{59}$  tagged autologous plasma. ••• Experimental plasma  $\text{Fe}^{59}$  data. ○-○-○ Experimental pleural fluid  $\text{Fe}^{59}$  data.  $\Delta$ - $\Delta$ - $\Delta$  Experimental edema fluid  $\text{Fe}^{59}$  data.

including four normal volunteers. Comparison of pool sizes and amounts of iron leaving the reduced three-pool system of the "preerythropoietic phase," through various pools per unit time, calculated on the basis of four different three-pool

models are indicated. The red cell iron turnovers and hemoglobin renewal rates are calculated on the basis of the initial reduced one-, two- and three-pool systems respectively. With better approximations to the plasma  $\text{Fe}^{59}$  disappearance data (i.e., extending the analysis from one- to two- to three-pools) the red cell

FIG. 16. Subject A.C. (Kimmelstiel-Wilson Disease): Radioactivity in ascitic fluid and in plasma after intraperitoneal injection of  $\text{Fe}^{59}$  bound to autologous ascitic fluid. ••• Experimental ascitic fluid  $\text{Fe}^{59}$  data. ○-○-○ Experimental plasma  $\text{Fe}^{59}$  data (multiplied by 10).

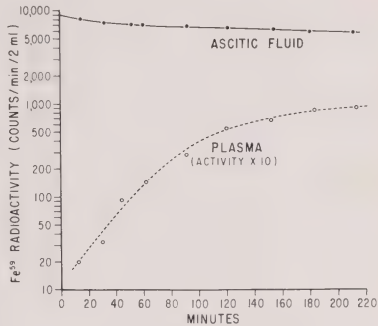


TABLE IX

Comparative values (in  $\mu\text{g}$  per 100 ml) for iron concentration and total iron binding capacity in plasma, and thoracic duct lymph in humans and dogs

Subject	Plasma Iron	Lymph Iron	Plasma TIBC	Lymph TIBC
Human, J. B. ....	128	75		103
Human, M. S. ....	55	80	270	263
Dog #3161 .....	177	186	347	345
Dog #3234 .....	250	245	324	336
Dog #3249 .....	280	300	441	430
Dog #3637 .....	70	54	398	258
Dog #4369 .....	52	53	306	280
Dog #5160 .....	90	87	229	211
		109*		230*
Dog #5523 .....	51	56	342	240
		148*		

\* Cervical lymph.

iron turnover values tend to decrease. The three-pool analysis gives results that are physiologically most tenable. In multiple-pool analysis of ferrokinetic data an inherent assumption is that the subjects are metabolically in a steady state (8,14). Though most patients show definite hematologic changes over a long period of time, during the short experimental interval (one to three weeks) they may be considered to be in a steady state. However, this may not hold true for W.O. This patient suffered episodes of acute congestive heart failure and required

TABLE X

*Dog 3161. Experimental plasma  $Fe^{59}$  disappearance and lymph  $Fe^{59}$  appearance and disappearance data*

Time after $Fe^{59}$ injection (min)	Activity in plasma in cpm/2 ml	Activity in lymph in cpm/2 ml
5	2705	
10	2625	25
15	2505	56
20	2445	
30	2235	213
45	2000	321
60	1845	353
75		376
90	1535	358
105		390
120	1235	283
135		287
150		333
180	830	364
240	572	225
300	397	144
340	306	
350		166

TABLE XI

*Dog 3249. Experimental plasma  $Fe^{59}$  disappearance and lymph  $Fe^{59}$  appearance and disappearance data*

Time after $Fe^{59}$ injection (min)	Activity in plasma in cpm/2 ml	Activity in lymph in cpm/2 ml
5	1870	
10	1785	
15	1720	118
20	1570	
30	1510	284
45	1320	456
60		454
63	1150	
75		446
90	990	411
105		369
125		340
126	760	
150		290
175	540	234
250	302	161
299	201.4	
300		90.3
350	134.1	71.5



TABLE XII

Dog 3161

$II_{pl}^*$ : Approximation of experimental plasma  $Fe^{59}$  disappearance data (Table X) by means of two exponential functions.

$II_{evlp}^\dagger$ : Theoretical radioactivity in extravascular labile pool based upon  $II_{pl}$  and multiplied by an appropriate factor.

Time after $Fe^{59}$ injection (min)	$II_{pl}$			$II_{evlp}$	
	A $2500e^{-0.0061t}$	B $300e^{-0.020t}$	C A + B	D $e^{-0.0061t} - e^{-0.020t}$	E 1000D
5	2425	271	2695	0.0652	65
10	2352	246	2600	0.1321	132
15	2282	222	2505	0.1718	172
30	2082	165	2245	0.2840	284
45	1900	122	2020	0.3533	353
60	1776	90	1865	0.3923	392
90	1444	50	1495	0.4122	412
120	1202	27	1230	0.3902	390
180	834	8	840	0.3062	306
240	578	2	580	0.2231	223
300	401		401	0.1579	158
340	315.5		316	0.1262	126

\* pl = plasma.

† evlp = extravascular labile pool.

TABLE XIII

Dog 3249

$II_{pl}$ : Approximation of experimental plasma  $Fe^{59}$  disappearance data (Table XI) by means of two exponential functions.

$II_{evlp}$ : Theoretical radioactivity in extravascular labile pool based upon  $II_{pl}$  and multiplied by an appropriate factor.

Time after $Fe^{59}$ injection (min)	$II_{pl}$			$II_{evlp}$	
	A $1750e^{-0.00714t}$	B $200e^{-0.028t}$	C A + B	D $e^{-0.00714t} - e^{-0.028t}$	E 1000D
5	1689	174	1865	0.0955	96
10	1629	151	1780	0.1753	175
15	1572	131	1705	0.2414	241
20	1517	114	1630	0.2957	296
30	1413	86	1500	0.3755	376
45	1269	57	1325	0.4413	441
63	1116	34	1150	0.4664	466
90	920	16	935	0.4454	445
126	712	6	720	0.3773	377
175	501	2	503	0.2790	279
250	294	0	294	0.1668	167
299	207		207	0.1181	118
350	143.8		144	0.0822	82

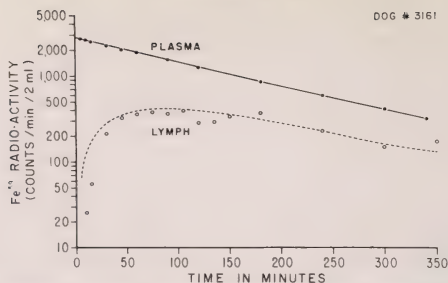


FIG. 17. Dog #3161 (Normal): Radioactivity in plasma and thoracic duct lymph after intravenous injection of  $\text{Fe}^{59}$  tagged autologous plasma. ••• Experimental plasma  $\text{Fe}^{59}$  data. ○○○ Experimental lymph  $\text{Fe}^{59}$  data. — Approximation of the experimental plasma radioactivity data by a sum of two exponential functions (i.e., theoretical values based on an initial two-pool system):

$$q_{pl}^* = 300e^{-0.020t} + 2500e^{-0.0061t}$$

---- Theoretical radioactivity in the second (extravascular labile) pool based upon the approximation of plasma radioactivity by two exponential functions, and multiplied by an appropriate constant:

$$q_{evlp}^\dagger = 1000 (e^{-0.0061t} - e^{-0.020t}).$$

\* pl = plasma.

† evlp = extravascular labile pool.

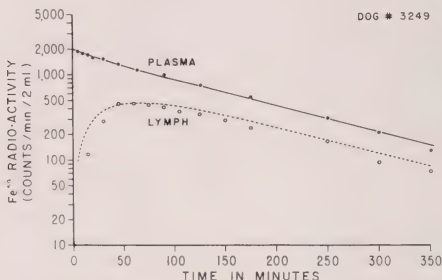


FIG. 18. Dog #3249 (Normal): Radioactivity in plasma and thoracic duct lymph after an intravenous injection of  $\text{Fe}^{59}$  tagged autologous plasma. ••• Experimental plasma  $\text{Fe}^{59}$  data. ○○○ Experimental lymph  $\text{Fe}^{59}$  data. — Approximation of the experimental plasma radioactivity data by a sum of two exponential functions (i.e., theoretical values based on an initial two-pool system):

$$q_{pl} = 200e^{-0.028t} + 1750e^{-0.00714t}$$

---- Theoretical radioactivity in the second (extravascular labile) pool, based upon the approximation of plasma radioactivity two exponential functions, and multiplied by an appropriate constant:

$$q_{evlp} = 1000 (e^{-0.00714t} - e^{-0.028t}).$$

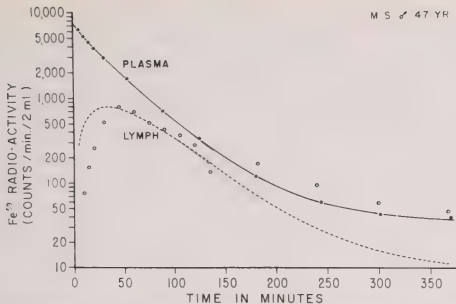


FIG. 19. Subject M.S. (Mild Iron Deficiency Anemia): Radioactivity in plasma and thoracic duct lymph after intravenous injection of  $\text{Fe}^{59}$ -beta-1-globulin. ••• Experimental plasma  $\text{Fe}^{59}$  data. ○○○ Experimental lymph  $\text{Fe}^{59}$  data. — Approximation of the experimental plasma radioactivity data by a sum of three exponential functions (i.e., theoretical values based on an initial three-pool system of iron metabolism):

$$q_{pl} = 3000e^{-0.041t} + 4050e^{-0.022t} + 46e^{-0.00077t}$$

----- Theoretical radioactivity in the second (extravascular labile) pool, based upon the above approximation of experimental plasma radioactivity by three exponential functions, and multiplied by an appropriate constant:

$$q_{evlp} = 65(-52.406e^{-0.041t} + 52.204e^{-0.022t} + 0.202e^{-0.00077t}).$$

..... Theoretical plasma and extravascular labile pool data based on an initial two-pool system. Values preceding the dotted line (during the first 100 to 150 minutes) cannot be distinguished from those based on an initial three-pool system. For the exact theoretical values corresponding to the two-pool model see Tables XV and XVI.

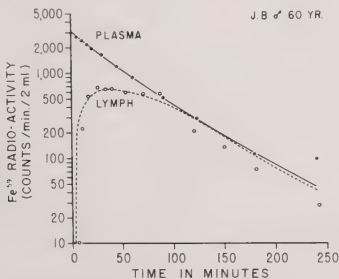


FIG. 20. Subject J.B. (Cirrhosis of the Liver): Radioactivity in plasma and thoracic duct lymph after an intravenous injection of  $\text{Fe}^{59}$ -beta-1-globulin. ••• Experimental plasma  $\text{Fe}^{59}$  data. ○○○ Experimental lymph  $\text{Fe}^{59}$  data. — Approximation of the experimental plasma radioactivity data by a sum of two exponential functions (i.e., theoretical values based on an initial two-pool system of iron metabolism):

$$q_{pl} = 1000e^{-0.041t} + 2100e^{-0.016t}$$

----- Theoretical radioactivity in the second (extravascular labile) pool, based upon approximation of experimental plasma radioactivity by two exponential functions, and multiplied by an appropriate constant:

$$q_{evlp} = 1900(e^{-0.016t} - e^{-0.041t}).$$

cardiotonic-diuretic therapy. To what extent this affected the ferrokinetic results is not known.

Iron destined for hemoglobin formation enters the immature cells of the erythron maturation pool from the marrow iron transit pool. After passing through several stages of maturation, the hemoglobin (and non-heme) iron is released into the circulating blood in young red cells. The average time elapsed between the disappearance of radioiron from the marrow iron transit pool and

TABLE XIV

*Subject M.S. (Mild Iron Deficiency Anemia). Experimental plasma  $Fe^{59}$  disappearance and lymph  $Fe^{59}$  appearance and disappearance data*

Time after $Fe^{59}$ injection (min)	Activity in plasma in cpm/2 ml	Activity in lymph in cpm/2 ml
5	6310	7
10	5300	76
15	5565	155
20	3840	253
30	2945	523
45		790
53	1705	
60		690
75		510
88	700	
90		425
105		360
120		280
124	330	
135		133
180	118	
182		164
240		91
244	58	
300		56
367	38	45
370		
1620	14.0	
2880	4.5	

its reappearance in the circulating blood is called the "time lag." This can be determined by superposition of the calculated theoretical red cell uptake curve and the corresponding experimental uptake curve as demonstrated in Fig. 27. The size of the erythron maturation pool (in terms of iron) can be expressed as the product of the time lag and the red cell iron turnover (8,31). Table XXVII gives such results for ten patients and four normal subjects.

#### DISCUSSION

Plasma  $Fe^{59}$  disappearance data have been extensively used for quantitative evaluation of erythropoiesis (4,6,7,8,12,19,32,33). During the first two or three

days after intravenous injection of  $\text{Fe}^{59}$ , tracer iron behavior in plasma can be best approximated by a sum of three exponential functions which indicates the existence of three mutually interchanging iron pools (8,14,15,17), plasma (first pool) and two other pools (second and third pools). The third iron pool has been recognized as the labile pool most intimately connected with erythropoiesis (7,8,

TABLE XV

*Subject M.S. (Mild Iron Deficiency Anemia). Approximations of experimental plasma  $\text{Fe}^{59}$  disappearance data (Table XIV) by means of two (II) and three (III) exponential functions respectively*

Time after $\text{Fe}^{59}$ injection (min.)	II			III			
	A $3000e^{-0.441t}$	B $4100e^{-0.021t}$	C A + B	D $3000e^{-0.041t}$	E $4050e^{-0.022t}$	F $40e^{-0.00077t}$	G D + E + F
5	2407	3691	6100	2444	3628	45.8	6120
10	1932	3323	5255	1990	3250	45.6	5285
15	1550	2992	4540	1622	2912	45.5	4580
20	1244	2776	4020	1321	2608	45.3	3975
30	801	2184	2985	877	2093	45.0	3015
45				474	1505	44.4	2025
53	291	1347	1640	341	1262	44.1	1645
60				256	1082	43.9	1380
75				139	778	43.4	960
88	62	646	710	81	584	43.0	710
90				75	559	42.9	675
105				40.5	401	42.4	485
120				22.0	289	41.9	355
124	13	303	316	18.6	265	41.8	325
135				11.7	207.8	41.5	261
180				1.8	77.4	40.0	119.2
182				1.8	73.7	40.0	115.5
240					20.7	38.2	58.9
244					19.0	38.12	57.3
300					5.67	36.51	42.2
302					5.27	34.46	39.7
367					1.22	34.67	35.9
370					1.22	34.60	35.8
1620						13.21	13.2
2880						5.01	5.0

34) and is here denoted as the "marrow iron transit pool" (Fig. 8). The identification and significance of the second pool has received only cursory attention. Pollycove and Mortimer (7) discuss the plasma  $\text{Fe}^{59}$  disappearance data in normal subjects both in terms of three and in terms of only two distinct underlying pools corresponding to an approximation of such data for a period of up to two weeks by sums of three and of two exponential functions respectively. None of our data for a period extending beyond the first day, could be adequately approximated by only two exponential functions. It was, therefore, concluded

TABLE XVI

Subject M.S. (Mild Iron Deficiency Anemia). Theoretical radioactivity in "the second pool" based upon analysis of plasma  $\text{Fe}^{59}$  disappearance data (Table XIV), in terms of two (II) and three (III) pool systems respectively, multiplied by appropriate constants

Column C:  $-52.406e^{-0.041t} + 52.204e^{-0.022t} + 0.202e^{-0.00077t}$

Time after $\text{Fe}^{59}$ injection (min)	II		III	
	A $e^{-0.231t} - e^{-0.031t}$	B 2900 A	C (See caption)	D 65C
5	0.0978	284	4.27	277
10	0.1666	484	7.32	475
15	0.2130	618	9.39	610
20	0.2422	703	10.74	696
30	0.2655	770	11.86	770
45	0.2506	727	11.36	737
53	0.2315	672		
60	0.2113	614	9.64	626
75	0.1701	494	7.80	506
88	0.1367	397		
90			6.09	395
105			4.66	303
120			3.53	229
124	0.0697	202		
135			2.66	173
180	0.0224	65		
182			1.10	72
240			0.44	29
244	0.0060	17		
300			0.23	15
367			0.17	11

TABLE XVII

Subject J.B. (Cirrhosis of the Liver)

$II_{pt}$ : Approximation of experimental plasma  $\text{Fe}^{59}$  disappearance data (Table XVIII) by means of two exponential functions.

$II_{evlp}$ : Theoretical radioactivity in extravascular labile pool based upon  $II_{pt}$  and multiplied by an appropriate factor.

Time after $\text{Fe}^{59}$ injection (min)	$II_{pt}$			$II_{evlp}$	
	A $2100e^{-0.016t}$	B $1000e^{-0.001t}$	C A + B	D $e^{-0.016t} - e^{-0.011t}$	E 1900D
5	1939	814.6	2755	0.1085	206
10	1789	663.6	2455	0.1885	358
15	1652	540.6	2195	0.2460	467
20	1525	440.5	1965	0.2856	542
30	1300	292.3	1590	0.3265	620
45	1022	158.0	1180	0.3287	624
60	804	85.4	890	0.2975	565
90	498	25.0	523	0.2119	403
123	293	6.4	299	0.1333	254
179	119.7	0.7	120.4	0.0563	107
240	45.2		45.2	0.0215	41
300	17.4		17.4	0.0082	15

TABLE XVIII

*Subject J.B. (Cirrhosis of the Liver). Experimental plasma  $Fe^{59}$  disappearance and lymph  $Fe^{59}$  appearance and disappearance data*

Time after $Fe^{59}$ injection (min)	Activity in plasma in cpm/2 ml	Activity in lymph in cpm/2 ml
5	2635	9.7
10	2440	
11		236
15	2180	
17		538
20	1905	
26.5		683
30	1655	
33.5		646
40.5		657
45	1195	
53.5		588
57.5		571
60	882	
88		563
90	507.4	
120		206.4
123	287.6	
150		131.1
179	109.8	
183		72.7
240	95.3	
242		37.3

TABLE XIX

*Subject I.H. (Diagnosis Unknown). Lymph  $Fe^{59}$  appearance and disappearance data following an intravenous injection of  $Fe^{59}$  beta-1-globulin*

Time after $Fe^{59}$ injection (min)	Lymph radioactivity in cpm/2 ml
29	87
49	116
59	160
69	186
79	189
89	199
109	332
129	349
149	450
169	493
189	449
209	509
229	533



TABLE XX

*Subject J.C. (Diagnosis Unknown). Lymph  $Fe^{59}$  appearance and disappearance data after intravenous injection of  $Fe^{59}$ -beta-1-globulin*

Time after $Fe^{59}$ injection (min)	Lymph radioactivity in cpm/2 ml
10	211
21	309
33	176*
46	159*
59	230
75	148
85	149
112	141
131	137
171	137
211	124
246	114
285	97
374	59
1467	33.6

\* lymph specimens clotted.

TABLE XXI

*Dog A (Normal). Plasma  $Fe^{59}$  disappearance and lymph  $Fe^{59}$  appearance and disappearance data after intravenous injection of  $Fe^{59}$  bound to autologous plasma*

Time after $Fe^{59}$ injection (min)	Plasma radioactivity in cpm/2 ml	Lymph radioactivity in cpm/2 ml
4.2	12,850	
7.5	11,850	
9.0	11,700	
11.0	11,450	14
14.0	11,250	
20.5		920
29.0	9,850	
31.0		2,200
44.5	8,180	
58.0	7,875	
99.0		1,030
117.0	4,560	
146.0	3,665	
148.0		1,020
180.0	2,820	
215.0	2,050	
218.0		925
260.0	1,335	750
301.0	875	
360.0	525	390

TABLE XXII

*Dog 3234 (Normal). Plasma  $Fe^{59}$  disappearance and lymph  $Fe^{59}$  appearance and disappearance data following intravenous injection of  $Fe^{59}$  bound to autologous plasma*

Time after $Fe^{59}$ injection (min)	Radioactivity in plasma in cpm/2 ml	Radioactivity in lymph in cpm/2 ml
5	2415	
10	2320	
15	2255	86
20	2175	
30	2115	230
45		305
60	1850	280
75		320
120	1375	360
150		365
180	1005	315
240	675	172
300		164
326	470	
358	395	116

TABLE XXIII

*Dog II (Normal). Plasma  $Fe^{59}$  disappearance and lymph  $Fe^{59}$  appearance and disappearance data following intravenous injection of  $Fe^{59}$  bound to autologous plasma*

Time after $Fe^{59}$ injection (min)	Plasma radioactivity cpm/2 ml	Lymph radioactivity cpm/2 ml
4.5		117
5	3755	
10	3435	
15	3165	263
20	2865	375
30	2460	590
45	1835	635
60	1380	585
75		520
90	775	375
105		320
120	450	325
135		310
150	275	
151		257
165		207
180	182	170
195		154
211		139
225		125
240	122	107
270		89.4
300		55.6
360		35.8

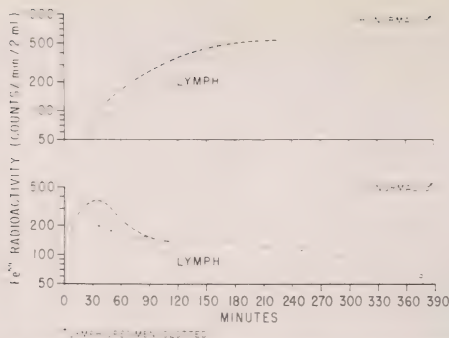


FIG. 21. Top. Subject L.H. (Diagnosis Unknown): Radioactivity in thoracic duct lymph after intravenous injection of  $\text{Fe}^{59}$ -beta-1-globulin.  $\circ-\circ-\circ$  Experimental lymph  $\text{Fe}^{59}$  data.

FIG. 21. Bottom. Subject J.C. (Diagnosis Unknown): Radioactivity in thoracic duct lymph after intravenous injection of  $\text{Fe}^{59}$ -beta-1-globulin.  $\circ-\circ-\circ$  Experimental lymph  $\text{Fe}^{59}$  data.

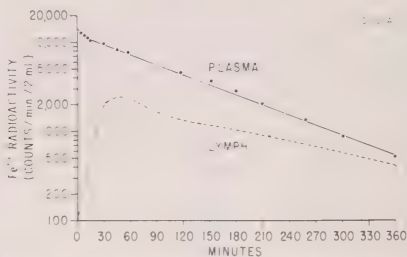


FIG. 22. Dog A (Normal): Radioactivity in plasma and thoracic duct lymph after intravenous injection of  $\text{Fe}^{59}$  bound to autologous plasma.  $\bullet-\bullet-\bullet$  Experimental plasma  $\text{Fe}^{59}$  data.  $\circ-\circ-\circ$  Experimental lymph  $\text{Fe}^{59}$  data.

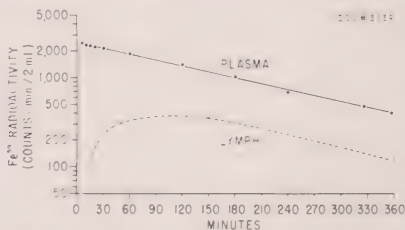


FIG. 23. Dog 3234 (Normal): Radioactivity in plasma and thoracic duct lymph after intravenous injection of  $\text{Fe}^{59}$  bound to autologous plasma.  $\bullet-\bullet-\bullet$  Experimental plasma  $\text{Fe}^{59}$  data.  $\circ-\circ-\circ$  Experimental lymph  $\text{Fe}^{59}$  data.

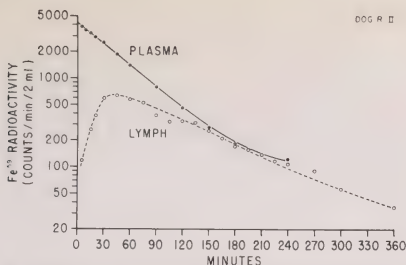


FIG. 24. Dog R.II (Normal): Radioactivity in plasma and thoracic duct lymph after an intravenous injection of  $\text{Fe}^{59}$  bound to autologous plasma. --- Experimental plasma  $\text{Fe}^{59}$  data. --- Experimental lymph  $\text{Fe}^{59}$  data.

TABLE XXIV

Pool sizes, iron turnovers and iron transfer rates on four normal volunteers (E. A.; J. O.; H. H.; A. T.) and the subject S. H. (Cerebral accident with normal peripheral hemogram) calculated on the basis of four different initial three-pool models (IIa, IIIa, IIIb and IVa) (17)

Three-Pool Model Considered†	Subject	Amount Fe (mg) in Each Pool $Q_i^*$ :			Amount Fe (mg/d) Leaving the Preerythropoietic System Through Each Pool $Q_i$ :			Total Iron Turn-over (mg/d) in Each Pool $Q_i$ :			Amount Fe (mg/d) Transferred From the $i$ th to the $j$ th Pool: $Q_i \rightarrow Q_j$ :					
		$Q_1$	$Q_2$	$Q_3$	$E_1$	$E_2$	$E_3$	$Q_1$	$Q_2$	$Q_3$	$Q_1 \rightarrow Q_2$	$Q_2 \rightarrow Q_1$	$Q_1 \rightarrow Q_3$	$Q_3 \rightarrow Q_1$	$Q_2 \rightarrow Q_3$	$Q_3 \rightarrow Q_2$
IIa	E.A.	1.6	4.0	44.00		3.3	18.9	25.9	52.6	45.6	25.9	3.7	0	0	45.6	26.7
	J.O.	2.3	4.4	37.00		4.2	16.8	22.9	45.1	39.1	22.9	1.9	0	0	39.0	22.3
	H.H.	2.2	2.9	46.10		3.6	15.3	23.2	37.7	29.7	23.2	4.4	0	0	29.7	14.4
	A.T.	3.1	4.3	118.90		5.3	24.2	39.7	70.1	54.6	39.7	10.2	0	0	54.6	30.4
	S.H.	2.5	12.8	24.80		4.2	13.3	28.5	47.4	32.2	28.5	11.0	0	0	32.2	18.5
IIIa	E.A.	1.6	0.37	42.80		3.3	18.9	25.9	5.0	20.9	5.0	1.7	20.9	2.0	0	0
	J.O.	2.3	0.47	35.10		4.2	16.8	22.9	5.1	17.8	5.1	0.9	17.8	1.0	0	0
	H.H.	2.2	0.46	43.80		3.6	15.3	23.2	6.2	17.0	6.2	2.6	17.0	1.8	0	0
	A.T.	3.1	0.66	112.00		5.3	24.2	39.7	11.0	28.8	11.0	5.7	28.8	4.6	0	0
	S.H.	2.5	2.1	29.40		4.2	13.3	28.5	8.9	19.6	8.9	4.7	19.6	6.3	0	0
IIIb	E.A.	1.6	5.3	42.80		47.7	18.9	25.9	71.5	20.9	5.0	23.9	20.9	2.0	0	0
	J.O.	2.3	11.8	35.10		106.0	16.8	22.9	127.8	17.8	5.1	21.8	17.8	1.0	0	0
	H.H.	2.2	3.8	43.80		29.6	15.3	23.2	51.1	17.0	6.2	21.5	17.0	1.8	0	0
	A.T.	3.1	4.1	112.00		33.1	24.2	39.7	68.3	28.8	11.0	35.2	28.8	4.6	0	0
	S.H.	2.5	9.8	29.40		20.0	13.3	28.5	42.2	19.6	8.9	22.2	19.6	6.3	0	0
IVa	E.A.	1.6	3.5	40.8	3.3	0	18.9	25.9	45.9	42.2	22.6	3.7	0	0	42.2	23.3
	J.O.	2.3	3.6	33.1	4.2	0	16.8	22.9	36.9	35.0	18.7	1.9	0	0	35.0	18.2
	H.H.	2.2	2.4	42.7	3.6	0	15.3	23.2	31.8	27.5	19.6	4.4	0	0	27.4	12.2
	A.T.	3.1	2.4	110.1	5.3	0	24.2	39.7	39.7	50.5	34.4	6.7	0	0	33.0	26.3
	S.H.	2.5	10.9	22.6	4.2	0	13.3	28.5	40.4	29.4	24.3	11.0	0	0	29.4	16.1

† For exact interconnections of these models see Figure 25.

\*  $Q_1$  = plasma iron pool.

$Q_2$  = extravascular labile (interstitial) iron pool.

$Q_3$  = marrow iron transit pool.

For exact definitions see Figures 1 and 2.

that the second or extravascular labile pool was significant and could not be neglected (Figs. 6, 8). Analysis of *in vivo* data in terms of this second pool (Fig. 11) indicated that such a compartment must have a generalized distribution. This was consistent with the hypothesis that the extravascular labile pool

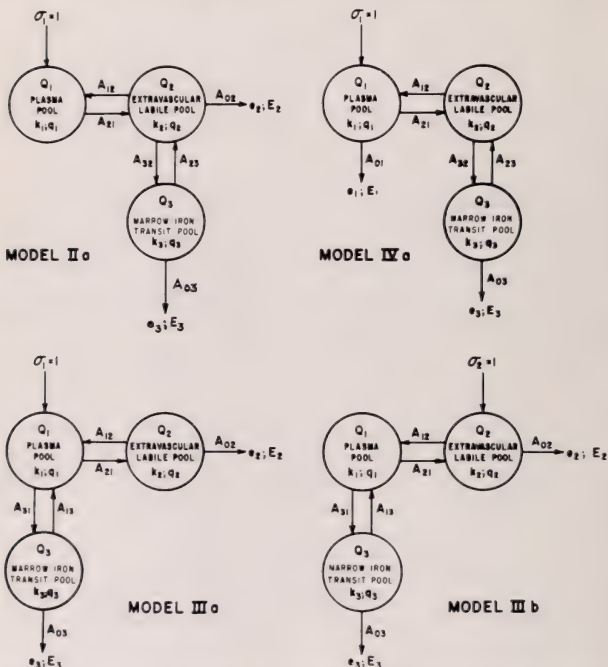


Fig. 25. Four three-pool models of the initial "preerythropoietic phase" of iron metabolism.  $\sigma_1$  is the fraction of total metabolite entering the system which enters it through pool  $Q_1$ . For the definitions of the other symbols see Figs. 1 and 2.

consisted of iron in the interstitial fluid. Evidence for this concept was found in studies on pathologic accumulations of interstitial fluids (edema, pleural and ascitic fluids), and in the interchange of radioiron between these compartments and plasma (Figs. 13, 14, 15, 16, Tables IV, V, VI, VII, VIII). No attempts at mathematical analysis or quantitation of the kinetics of iron movement were, or could be, made in these studies, since the conditions of the experiments precluded the existence of a steady state. Such fluids formed reservoirs of iron, where

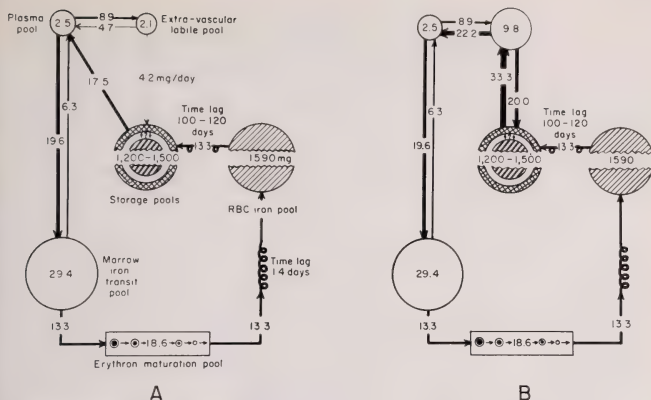
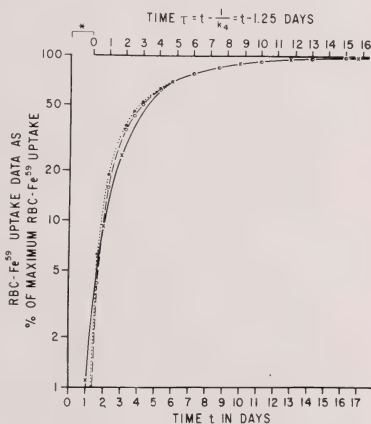


FIG. 26. Subject S.H.: Models of iron metabolism with quantitative results from Table XXIV for "preerythropoietic systems" IIIa and IIIb. The erythropoietic phase, circulating red cell pool and storage iron pool are discussed in the text below. All pool sizes and turnover values were measured or calculated except for the storage component which was obtained from the literature (1, 2).

FIG. 27. Subject A.M. (Normal): Experimental RBC  $\text{Fe}^{59}$  uptake data and their approximation by theoretical values derived from the three-pool analysis of plasma  $\text{Fe}^{59}$  disappearance data.  $\times-\times-\times$  Experimental RBC  $\text{Fe}^{59}$  uptake (lower time scale  $t$ ).  $\bullet-\bullet-\bullet$  Theoretical RBC  $\text{Fe}^{59}$  uptake for models IIIa and IIIb (upper time scale  $\tau = t - 1.25$  days).  $\circ-\circ-\circ$  Theoretical RBC  $\text{Fe}^{59}$  uptake for models IIa and IVa (upper time scale  $\tau = t - 1.25$  days).



\* The bracket,  $\frac{1}{k_4}$ , refers to time lag  $= \frac{1}{k_4} = 1.25$  days (17, 23).

† After day 5 all three lines coincide.

the hypothesis of instantaneous mixing could not be held as "approximately" true.

Because of these considerations, a study of lymph (8,35,36) as the only accessible normal sub-compartment of the interstitial fluid (37,38) was undertaken. The role of lymph, and therefore of interstitial fluid, as a possible medium for transport of iron has been investigated previously. In 1939 Moore et al. (39) demonstrated an increase in the iron content of thoracic duct lymph in dogs following the intravenous injection of large doses of iron. Peterson and Mann

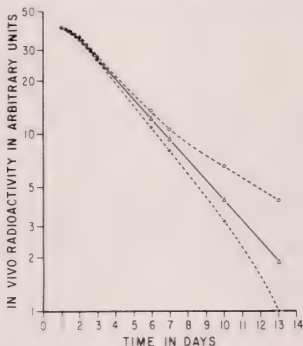


FIG. 28. Subject A.M. (Normal, with 99% RBC  $\text{Fe}^{59}$  uptake): Experimental in vivo radioactivity data (corrected for blood background) over the bone marrow area, and their approximation by theoretical values derived from three-pool analysis (Models IIIa and IIIb) of plasma  $\text{Fe}^{59}$  disappearance data.  $\circ$ - $\circ$ - $\circ$  Experimental in vivo radioactivity over bone marrow area, with 90%\* correction for blood background.  $\bullet$ - $\bullet$ - $\bullet$  Experimental in vivo radioactivity over bone marrow area, with 100% correction for blood background.  $\triangle$ - $\triangle$ - $\triangle$  Theoretical in vivo radioactivity over any given erythropoietic area (including activity in bone marrow iron transit pool and erythron maturation pool).

\* The rationale for the modified 90% blood background correction is that radioactivity distribution within the body may differ for plasma and for red cells due to differences between venous and local "organ hematocrits."

in 1952 (40), and subsequently Everett et al. (41) suggested that the most likely source of lymph iron was resorption from the blood stream.

The similarity of iron-binding proteins and of iron concentrations in plasma, in lymph, and in other interstitial fluids has been shown. Rapid interchange of radioiron between plasma and lymph was directly demonstrated from studies of plasma and of thoracic duct lymph after intravenous injection of  $\text{Fe}^{59}$ . The observed values in lymph, when compared to theoretical values of the second pool derived mathematically both on the basis of two- and of three-pool models, showed anticipated agreement. Some deviations from predicted values are to be expected because of the experimental conditions, in particular because of changes in the steady state (lymph was not recirculated). Lymph forms only a fraction



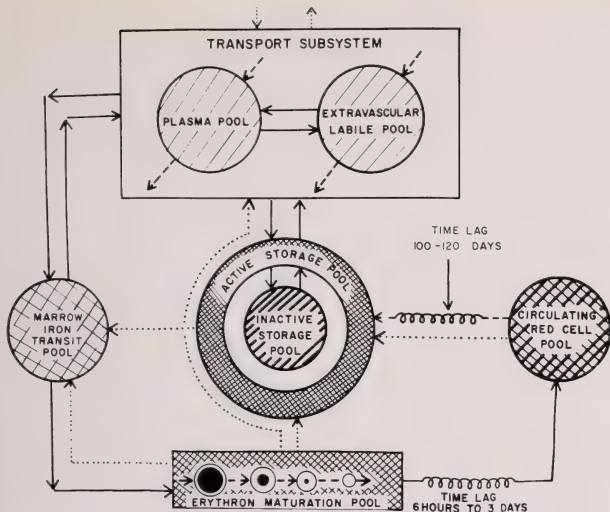


FIG. 29. A schematic (mathematical) representation of a multiple pool model of iron kinetics

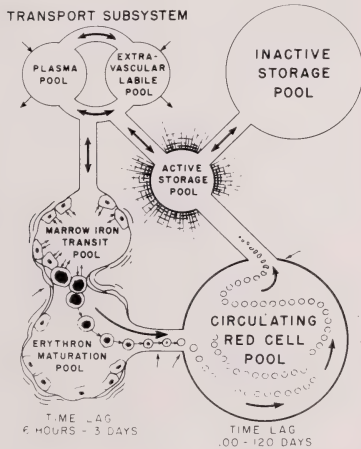


FIG. 30. A schematic ("physiological") representation of a multiple pool model of iron kinetics.

TABLE XXV

Comparison of pool sizes and amounts of iron leaving the reduced three pool system of the preerythropoietic phase, through various pools per unit time, calculated on the basis of the four three pool models (IIa, IIIa, IIb and IVa, Figure 25) for seventeen subjects including normals and patients with various blood disorders

Subject	Diagnosis	Plasma Iron Pool, $Q_p$ , (mg)				Extravascular Labile Iron Pool, ( $Q_e$ , mg)				Marrow Iron Transit Pool, ( $Q_m$ , mg)				"Iron to Storage" (mg/day)				Red Cell Iron Turnover, $f_0$ (mg/day)			
		Model IIa	Model IIb	Model IIIa	Model IIIb	Model IVa	Model IVb	Model IIIa and IVa	Model IIIb and IVb	Model IIa	Model IIb	Model IIIa	Model IIIb	$f_0$ (Model IIa)	$f_0$ (Model IIb)	$f_0$ (Model IIIa)	$f_0$ (Model IVa)	Model IIa	Model IIb	Model IIIa	Model IVa
E.A.	Normal Volunteer	1.62	1.0	0.37	5.3	3.5	44.0	42.8	40.8	42.8	40.8	42.8	40.8	3.3	47.7	47.7	18.9	47.7	47.7	47.7	47.7
J.O.	Normal Volunteer	2.32	1.1	0.47	11.8	3.6	37.0	35.1	33.1	37.0	35.1	37.0	35.1	4.2	106.0	106.0	16.8	106.0	106.0	106.0	106.0
H.H.	Normal Volunteer	2.21	2.9	0.46	3.8	2.4	46.1	43.8	42.7	46.1	43.8	46.1	43.8	3.6	29.6	29.6	15.3	29.6	29.6	29.6	29.6
A.T.	Normal Volunteer	3.14	4.3	0.66	4.1	2.4	118.9	112.0	110.1	118.9	112.0	118.9	112.0	5.3	33.1	33.1	24.2	33.1	33.1	33.1	33.1
S.H.	Cerebral Accident: Normal peripheral hemogram	2.51	12.8	2.1	9.8	10.9	24.8	29.4	22.7	24.8	24.8	29.4	22.7	4.22	20.0	20.0	13.3	20.0	20.0	20.0	20.0
A.M.	Neurological Disorder: Normal peripheral hemogram	4.27	11.6	0.45	2.8	11.5	45.9	54.6	45.7	45.9	45.9	54.6	45.7	0.15	0.93	0.93	14.9	0.93	0.93	0.93	0.93
E.R.	Respiratory Infection: Normal peripheral hemogram	2.47	2.1	0.30	0.82	2.1	25.1	26.3	25.1	25.1	25.1	26.3	25.1	0.00*	0.00*	0.00*	20.7	0.00*	0.00*	0.00*	0.00*
F.A.	Cirrhosis of Liver: Normal peripheral hemogram	3.98	10.8	3.7	6.6	9.9	58.1	60.0	54.7	58.1	58.1	60.0	54.7	3.74	6.35	6.35	9.8	6.35	6.35	6.35	6.35
G.B.	Polycythemia vera	2.55	5.7	0.89	2.5	5.3	59.6	60.1	56.5	59.6	59.6	60.1	56.5	4.83	13.7	13.7	26.2	13.7	13.7	13.7	13.7
C.W.	Polycythemia vera	1.22	3.3	0.86	2.5	8.1	20.9	42.8	37.2	20.9	20.9	42.8	37.2	0.00*	0.00*	0.00*	18.7	0.00*	0.00*	0.00*	0.00*
A.G.	Myeloid metaplasia	4.80	20.5	4.2	15.2	14.1	23.3	28.5	17.1	23.3	23.3	28.5	17.1	27.0	98.2	98.2	12.5	98.2	98.2	98.2	98.2
L.B.	Myeloid metaplasia (spent polycythemia vera)	7.53	7.1	3.0	4.5	5.9	59.1	53.4	50.1	59.1	59.1	53.4	50.1	21.0	31.9	31.9	8.6	31.9	31.9	31.9	31.9
L.L.	Myeloid metaplasia (spent polycythemia vera)	1.85	12.7	4.3	5.6	12.3	42.4	48.7	41.5	42.4	42.4	48.7	41.5	3.50	4.57	4.57	17.5	4.57	4.57	4.57	4.57
M.A.T.	Myeloid metaplasia	2.42	14.2	2.3	7.0	12.6	31.5	37.7	28.9	31.5	31.5	37.7	28.9	5.72	17.3	17.3	16.6	17.3	17.3	17.3	17.3
B.R.	Myeloid metaplasia	3.01	24.3	4.4	15.2	20.7	50.5	59.2	45.6	20.7	20.7	59.2	45.6	6.93	23.9	23.9	17.2	23.9	23.9	23.9	23.9
R.M.	Myeloid metaplasia	5.10	92.2	0.98	9.8	80.3	40.7	428.	349.	80.3	80.3	40.7	349.	29.9	29.9	29.9	44.7	29.9	29.9	29.9	29.9
F.E.	Hemolytic anemia with hemosiderosis	9.35	103.0	14.3	74.6	67.5	147.	160.	101.	67.5	67.5	147.	160.	79.5	414.	414.	32.8	414.	414.	414.	414.
C.L.	Hemolytic anemia	8.40	39.4	15.1	24.5	33.0	95.4	102.	82.8	33.0	33.0	95.4	102.	27.6	44.5	44.5	18.4	44.5	44.5	44.5	44.5
A.M.	Treated iron deficiency anemia	0.814	40.2	1.0	1.8	3.9	17.3	19.3	17.1	3.9	3.9	17.3	19.3	0.62	1.11	1.11	9.7	1.11	1.11	1.11	1.11
W.O.	Hemochromatosis	6.85	10.1	4.1	6.5	7.8	59.3	60.1	55.7	7.8	7.8	59.3	60.1	6.45	10.2	10.2	13.6	10.2	10.2	10.2	10.2
S.S.	Aplastic anemia	8.26	3.8	1.3	2.3	2.8	28.1	22.8	20.8	2.8	2.8	28.1	22.8	12.2	22.4	22.4	1.4	22.4	22.4	22.4	22.4

\* The "iron to storage" value of zero corresponds to an experimentally measured fractional radioiron red cell incorporation of 1.00. This measurement has inherent experimental errors which may obscure the "iron to storage" component. This component is a function of  $1 - f$  where  $f$  = fractional red cell radioiron uptake. Therefore, for values of  $f$  close to 1.00, a small relative error in  $f$  corresponds to a larger

TABLE XXVI

*Comparison of red cell iron turnover rates (and hence of hemoglobin renewal) calculated on the basis of a a) one-pool system; b) two-pool\* system; c) three-pool\* system, on twenty-one subjects including normals and patients with various blood dyscrasias*

Subject	Diagnosis	Weight (kg)	RBC Cr <sup>51</sup> Volume (ml)	U Maximum (Fractional) Red Cell Fe <sup>59</sup> Uptake	Red Cell Iron Turnover (mg/day) Based Upon			Percent Hemoglobin Renewal/Day Based Upon		
					One-pool Analysis	Two-pool Analysis	Three-pool Analysis	One-pool Analysis	Two-pool Analysis	Three-pool Analysis
E.A.	Normal Volunteer	57.7	1840	0.85	22.6	20.7	18.9	1.23	1.12	1.03
J.O.	Normal Volunteer	63.6	2035	0.80	18.2	17.7	16.8	0.90	0.87	0.82
H.H.	Normal Volunteer	62.3	1860	0.81	20.1	16.8	15.3	1.08	0.90	0.82
A.T.	Normal Volunteer	88.2	2230	0.82	30.0	26.6	24.2	1.35	1.19	1.09
S.H.	Cerebral Accident:	81.5	1590	0.759	20.5	17.9	13.3	1.29	1.13	0.83
	Normal peripheral hemogram									
A.M.	Neurological Disorder:	70.6	1695	0.990	24.1	22.5	14.9	1.41	1.33	0.88
	Normal peripheral hemogram									
E.R.	Respiratory Infection:	71.6	1900	1.000	32.9	28.8	20.7	1.73	1.51	1.09
	Normal peripheral hemogram									
F.A.	Cirrhosis of Liver:	62.3	1200	0.725	34.3	20.9	9.8	2.86	1.74	0.82
	Normal peripheral hemogram									
G.B.	Polycythemia vera	82.7	3955	0.845	47.8	39.0	26.2	1.21	0.99	0.66
C.W.	Polycythemia vera	52.3	2170	1.000	39.1	27.0	18.7	1.80	1.24	0.86
A.G.	Myeloid metaplasia	47.4	1250	0.316	28.8	19.3	12.5	2.30	1.54	1.00
L.B.	Myeloid metaplasia (spent polycythemia vera)	63.6	1700	0.292	27.4	17.2	8.6	1.61	1.01	0.51
L.L.	Myeloid metaplasia (spent polycythemia vera)	71.8	2970	0.833	118.5	59.8	17.5	3.99	2.01	0.59
M.A.T.	Myeloid metaplasia	62.7	1210	0.744	36.0	25.2	16.6	2.97	2.08	1.37
B.R.	Myeloid metaplasia	77.3	1530	0.713	31.5	27.3	17.2	2.06	1.78	1.12
R.M.	Myeloid metaplasia	54.5	2155	0.599	138.5	130.2	44.7	6.43	6.04	2.08
F.E.	Hemolytic anemia with hemosiderosis	73.2	1540	0.292	64.9	48.6	32.8	4.21	3.15	2.13
C.L.	Hemolytic anemia	76.8	1320	0.400	67.2	36.5	18.4	5.09	2.76	1.39
A.M.	Treated iron deficiency anemia	61.8	993	0.940	30.6	15.2	9.7	3.08	1.53	0.98
W.O.	Hemochromatosis	53.0	1760	0.678	38.7	22.5	13.6	2.20	1.28	0.77
S.S.	Aplastic anemia	56.4	768	0.105	3.1	2.9	1.4	0.41	0.38	0.19

\* Red cell iron turnover and hemoglobin renewal rates are the same for all models considered.

of the interstitial fluid (37,38) and, therefore, of the extravascular labile pool. It is probable that the stable iron concentration, and hence radioactivity per unit volume of interstitial fluid may vary with different subcompartments. While it has been demonstrated (8,42) that under appropriate conditions a number of separate but related subcompartments may exhibit the mathematical tracer behavior of a single pool, some variation, nevertheless, may be anticipated in tracer behavior of any individual subcompartment. Hence, the experimental tracer behavior in lymph should follow, at best, only a general theoretical pattern of the second or extravascular labile pool. These predicted findings, experimentally confirmed, substantiate the hypothesis that the second-pool rapidly interchanging with plasma is iron in interstitial fluid.

TABLE XXVII  
*Erythron maturation iron pools: Calculations*

Subject*	Red cell iron turnover (mg/day) (three-pool analysis)	×	Estimated† time lag (days)	=	Erythron maturation pool (mg iron)
E.A.	18.9		2.00		37.8
J.O.	16.8		1.25		21.0
H.H.	15.3		1.65		25.2
A.T.	24.2		1.10		26.6
S.H.	13.3		1.40		18.6
A.M.	14.9		1.25		18.6
F.A.	9.8		1.67		16.4
C.W.	18.7		1.00		18.7
A.G.	12.5		0.70		8.7
R.M.	44.7		1.00		45.0
C.L.	18.4		2.40		44.0
A.M.	9.7		1.60		15.5
W.O.	13.6		1.00		13.6
S.S.	1.4		1.50		2.1

\* For diagnosis see Table XXV.

† Estimate for time lag is incorrect when significant hemolysis is present.

The apparent slow interchange of plasma transferrin with extracellular fluid led Pollycove and Mortimer (7) to conclude that the movement of iron into extracellular fluid was also slow, amounting to but one mg per day. Pollycove and Mortimer's three-pool model for normal subjects (7) is mathematically equivalent to model IIIa (8,17). Physiologically, the concept of the "labile erythropoietic pool" may correspond to the "third" or marrow iron transit pool. The interpretation of the second iron pool as a "labile storage pool," (7) however, is no longer feasible in view of the demonstration of early and significant iron interchange between plasma and interstitial fluid. Table XXIV gives the transfer rates between these compartments. The rates are considerably larger than one mg per day for all models considered. The quantity of interstitial fluid iron varies with the three-pool model considered (Table XXV) and may be either smaller or greater than the corresponding total plasma iron. For a given subject this

variation in the calculated interstitial fluid iron may be almost 100-fold (patient R.M.). Similar variations in the size of the second pool result when the initial 4 to 8 hour plasma  $\text{Fe}^{59}$  data are analyzed in terms of two-pool models (12,14). At present, there is no direct method of measuring the total iron content in interstitial fluid. Since iron in both plasma and interstitial fluid is bound to the same protein, determination of the interstitial siderophilin pool would afford a means of measuring the corresponding iron content (assuming the iron saturation of interstitial siderophilin to be known). Plasma and interstitial fluid transferrin pools have been reported to be approximately equal in size (43,44). These results were based upon extrapolations of plasma disappearance curves and may be valid for some particular pool model (not necessarily the same for each subject). Such extrapolations, however, cannot be used as a general method for calculating pool sizes (Table XXV). Katz (43) and Awai and Brown (44) noted that radioiodinated transferrin disappears from plasma at a slower rate than radioiron. These observations do not imply any mechanism for the transfer of iron from plasma to the other compartments of the "preerythropoietic phase" of iron metabolism; they neither confirm nor reject the hypothesis of the initial three-pool system of iron metabolism.

The marrow iron transit pool is that compartment of the "preerythropoietic phase" of iron metabolism which provides the iron destined for hemoglobin synthesis. This pool cannot be defined either spatially or chemically from available radioiron data. It may overlap or even coincide anatomically with the "erythron maturation pool"; it may be a reservoir of iron which envelops and bathes the cells of the erythron maturation pool. Its size depends upon the particular three-pool model of the preerythropoietic system used, and ranges from 33.1 to 118.9 mg in four normal subjects (Table XXV). The amount of iron leaving this pool for the erythron maturation pool (red cell iron turnover) is identical in each patient for the four models considered (15,17).

The maximum fractional red cell  $\text{Fe}^{59}$  uptake,  $U$ , is one of the parameters required for the calculation of absolute iron transfer rates (Table XXIV), of all iron pool sizes (Tables XXIV, XXV) and of iron turnovers (Tables XXV, XXVI) (15,17). In patients with hemolytic disease the observed  $U$  is less than the "true" maximum due to the loss of circulating red cell  $\text{Fe}^{59}$  by random hemolysis. The true values are not known, and the observed  $U$ 's have been used in the calculations of the above tables thereby introducing systematic errors. Since the true uptakes lie between the observed values and 1.00, the extent of such errors is indicated in Table XXVIII where red cell iron turnovers are calculated both on the basis of observed  $U$  and of the maximum possible value, 1.00.

The "erythron maturation pool" is defined as iron in the hemoglobin of maturing red cells (normally in the marrow) which subsequently is released into the peripheral blood. This pool can be interpreted as an "infinite" series of maturing heme containing cells comprising (or as irreversible reactions characteristic of) the different stages of erythropoiesis, interposed between the marrow iron transit and the circulating red cell iron pools.

In iron kinetic studies the erythron maturation pool manifests itself as a time lag between the disappearance of radioiron from the "preerythropoietic phase"

and its subsequent reappearance in the peripheral blood. The values of both the time lag and the size of the erythron maturation pool are presented in Table XXVII. The estimated time lag on 14 subjects averages 1.4 days which is in good agreement with Pollycove's and Mortimer's (7) estimate of the mean effective hemoglobinization time of 1.4 days. The calculated iron content of the erythron maturation pool ranges from 2.1 mg in aplastic anemia to 45 mg in a subject with myeloid metaplasia, and is approximately 27.6 mg in normal subjects. The greatest error in calculating this pool size lies in estimating the value of the time lag; where hemolysis is present this error may be considerable. A more detailed discussion on the nature of the marrow iron transit pool and erythron maturation pool is presented in the third paper of this series (13).

TABLE XXVIII

*Pool sizes, iron turnovers and hemoglobin renewal rates; comparison of observed values and assumed values for red cell  $Fe^{59}$  incorporation in patients with hemolysis*

Patient	U	$Q_1$ (mg)	$Q_2$ (mg)		$Q_3$ (mg) IIIa = IIIb	$E_2$ (mg/day)		$E_3$ (mg/day) IIIa = IIIb	Hgb Renewal Rate (%) IIIa = IIIb	Erythron Maturation Pool (mg) IIIa = IIIb
			IIIa	IIIb		IIIa	IIIb			
R.M.	0.60 (observed)	5.10	0.98	9.8	428	29.9	299	45	2.1	45
	0.90 (assumed)	"	0.40	4.0	479	7.4	74	67	3.1	67
	1 (assumed)	"	0.21	2.1	495	0	0	75	3.5	75
F.E.	0.29 (observed)	9.35	14.3	74.6	160	79.5	414	414	2.1	66
	0.90 (assumed)	"	4.9	25.4	249	9.0	49	101	6.5	202
	1 (assumed)	"	3.6	18.7	261	0	0	112	7.3	225
A.G.	0.32 (observed)	4.80	4.2	15.2	28.5	27.0	98	12.5	1.5	8.7
	0.90 (assumed)	"	1.9	6.8	43	4.0	14.4	36	2.8	25
	1 (assumed)	"	1.5	5.4	46	0	0	40	3.6	28

Twenty-four to forty-eight hours after injection the distribution of radioiron within the "preerythropoietic system" approaches an equilibrium. The third exponential function of the approximating equation to plasma  $Fe^{59}$  disappearance data becomes predominant in all three component pools. The tracer behavior in plasma (Fig. 7), extravascular labile and marrow iron transit pools can now be expressed as  $e^{-\lambda_3 t}$  multiplied by a corresponding constant.  $\lambda_3$  is the rate with which radioactivity disappears from the "preerythropoietic system" after equilibrium is attained, and must not be confused with the relative rate,  $k_3$ , in the third pool.  $k_3$  is the fraction of total pool radioactivity (or total pool iron) leaving the marrow iron transit pool (for plasma, for interstitial fluid compartment as well as for the erythron maturation pool) per unit time;  $k_3$  does not take into account the radioiron returned to the third pool from the other component pools, and hence does not describe the tracer behavior within the marrow iron transit pool at any time.



The exponential function,  $e^{-\lambda_3 t}$ , is characteristic not only of the tracer behavior in the "preerythropoietic" but also of the "posterythropoietic" phase. Thus, the same exponential component appears in the analysis (Figs. 12, 27, 28). This systematic appearance of the same slope as a predominant component of plasma radioiron clearance, as well as of the radioactivity over all body sites and (with a negative coefficient) in red cell  $\text{Fe}^{59}$  uptake data, signifies that all iron tracer behavior is affected by the same reduced three-pool system. This emphasizes the unity of the multiple-pool approach to iron kinetics, and leads to the schematic representations of iron metabolism as shown in Figs. 29 and 30 (13). The dynamics of absorption and excretion of iron and of its utilization for enzyme and myoglobin synthesis are not considered except as general entry into and exit from the depicted system. Plasma and extravascular labile iron pools are jointly denoted as the "transport subsystem" in accordance with their physiological functions. The exact interconnections between the pools of the "preerythropoietic phase" of iron metabolism are not known, as indicated.

The "posterythropoietic phase" of iron metabolism consists of red cell and of storage compartments. After a time lag of up to three days, reticulocytes and mature red cells are released from the erythron maturation pool into the circulating blood where they normally remain for a finite period of time. The process of senescence of circulating red cells can be considered as an infinite sequence of unidirectionally connected progressively aging cells, manifesting itself as another time lag of 100 to 120 days (Figs. 29, 30). At the end of this process red cells are sequestered within the reticulo-endothelial system. The iron (derived from these sequestered cells) is now within the reticulo-endothelial system in an "active storage" compartment and most of it is returned (45) to the transport subsystem.

In normal subjects the red cell radioiron uptake is about 90%. This permits a direct "inactive storage" deposition of about 10% of the injected radioiron. Normally "inactive storage" iron in its various forms and especially as ferritin is not inert (7,46,47). Radioiron initially transferred to storage pools must eventually return, at least in part, to the transport subsystem. Theoretically, in a steady state system, such movements should manifest themselves by the presence of a fourth exponential component (with a half-time of disappearance of months) in the plasma  $\text{Fe}^{59}$  disappearance curve. This component, however, cannot be detected at present partly because radioactivity in plasma approaches background levels after three weeks. In body surface scanning such storage activity may be completely obscured by overcorrection for highly radioactive blood background and by the limitations of the experimental conditions. In patient F.A. (Fig. 12, Table XXVI) with a maximum red cell  $\text{Fe}^{59}$  uptake of 73%, the storage components over the spleen and over the liver could be detected.

The "iron to storage" (Table XXV) represents iron which enters the storage compartments (active and or inactive) directly from the transport subsystem. It does not include iron deposited from effete red cells, or absorbed iron which may be directly stored without prior entry into the general circulation (48). Therefore, it does not represent either total storage iron turnover or daily storage



iron deposition. For example, in hemochromatosis iron from senescent red cells may be a significant factor in total storage iron accumulation. This mechanism would not be reflected in the "iron to storage" values.

Metabolic pools do not necessarily form contiguous compartments; on the other hand, distinct metabolic pools do not have to be spatially separated from each other. In the case of erythrokinetics such pools overlap anatomically. Thus, plasma and red cell pools occupy approximately the same vascular spaces. Extravascular labile pools, storage pools, marrow iron transit and erythron maturation compartments overlap to various degrees.

This completes the description of the metabolism of iron in terms of multiple pool systems as derived from ferrokinetic studies. Such mathematical models represent "idealizations" (simplifications) of the metabolic processes considered, and should not contradict the accepted basic concepts of physiology. Above all such models are useful only if they elucidate metabolic processes beyond the mere representations of the experimentally available data.

#### SUMMARY

1. From theoretical and physiological considerations it has been postulated that interstitial fluid plays a significant part in the transport of iron.

2. Experimental verification of the significance of interstitial fluid as an iron compartment has been obtained from studies on lymph, various transudates and edema fluid as representative of interstitial fluid.

3. The individual significance and interrelationships of different iron pools are presented.

4. After equilibration, the tracer behavior in all pools, except storage pools, reflects the final course of the plasma  $\text{Fe}^{59}$  disappearance curve.

5. From this unity of tracer iron behavior in various pools, a mathematical model of iron kinetics has been developed.

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## Studies in Iron Kinetics

### II. Interpretation of Experimental Data in Terms of Multiple Pool Systems\*

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In our studies of iron kinetics, the method of multiple-pool analysis (1, 2, 3, 4, 5, 6, 7) is applied directly to the data obtained from in vitro measurements of plasma  $\text{Fe}^{59}$  disappearance, red cell  $\text{Fe}^{59}$  uptake, and to the tissue localization of the radioiron as determined by in vivo counting. The plasma  $\text{Fe}^{59}$  disappearance data during the six hours following the intravenous injection of a tracer dose can be approximated by a sum of two exponential functions, thus indicating an initial rapid interchange between the plasma iron pool and a second pool designated as the extravascular labile iron pool (1, 2, 5, 6) (Appendix A). Such a two-pool system can be represented by a variety of mathematical models (3) (Fig. 1). A convenient way of classifying these models is by means of the modes of exit and entry of the stable isotope from and into the two pools comprising the system. Four of the sixteen representative models (3) (Fig. 1) considered may be eliminated as working hypotheses because they lead to physiologically untenable hemoglobin renewal rates in normal subjects (1). Attempts to eliminate some of the other two-pool models by considering the calculated sizes of the corresponding extravascular labile pools, have been unsuccessful, mainly because of lack of independent information about the iron content of the extravascular labile compartment.

To obtain further insight into the dynamics of iron metabolism, serial in vivo observations were performed over different parts of the body. The early studies were based on two-pool analysis and emphasized, arbitrarily, splenic in vivo data. Subsequent studies were based on three-pool analysis and on simultaneous in vivo observations over spleen, liver and bone marrow (sacral area).

The in vivo counter in a given position over a specific region sees and registers the radioactivity derived from several distinct sources (Fig. 2). One such source is the  $\text{Fe}^{59}$  in the circulating blood, which may be called the blood background. During the first few hours after the injection, all such activity is contained in plasma and hence is proportional to the specific activity of the plasma pool. After a few days it is almost exclusively due to the iron incorporated in the newly formed red cells. The second source, which is the radioactivity in the

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extravascular labile pool, is transient, and is significant only during the first twelve hours of observation. A third source is derived from erythropoiesis,\* (which may be in adjacent erythropoietic sites). The fourth source may be referred to as the iron stores, and the fifth source consists of scattered radiation.

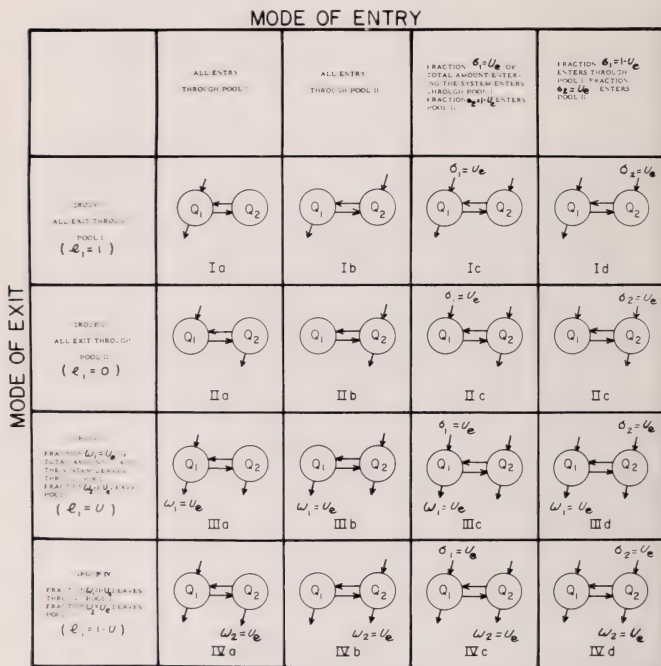


FIG. 1. Schematic representation of sixteen extreme two-pool models of iron metabolism, arranged according to the various modes of exit and entry of the iron.

Such sources as myoglobin, cytochrome, and other enzymes are probably of negligible significance in this context.

Immediately following the injection of a tracer dose of  $\text{Fe}^{59}$ , the counter registers circulating blood activity only, i.e., blood background; the correction for blood background at any subsequent time can be obtained by extrapolating both the gross in vivo counts over a given area and the whole blood in vitro

\* The marrow iron transit pool and the erythron maturation pool (5, 6).

counts to zero time, and then multiplying the resulting ratio by the corresponding whole blood counts (8) (Fig. 3). This correction is valid for the first few hours of the experiment and is adequate on subsequent days when the "peripheral venous" and "local" hematocrits do not differ significantly, i.e., when the changes in the radioactivity (either in plasma or in red cell mass) of the peripheral venous whole blood reflect the corresponding changes in the *in vivo* activity of the circulating component (see below). In some patients where the body hematocrit is considerably lower than the peripheral venous hematocrit, the above correction may be excessive after the first day, and a smaller ratio may have to be used at such times to avoid negative results (5, 7). In patients with marked splenomegaly, accompanied by frequent changes in spleen size, addi-

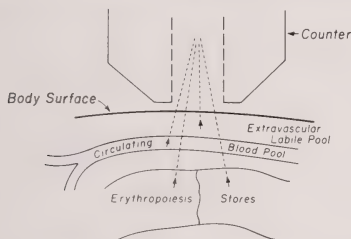


FIG. 2. The *in vivo* counter detects radioactivity derived from several distinct sources but cannot differentiate between the various origins of radiation, and registers all activity as a single set of events.

The five distinct sources of  $\text{Fe}^{59}$  radioactivity detected over the splenic site are:

- 1) The circulating blood (or blood background);
- 2) The extravascular labile pool;
- 3) The erythropoietic areas (such as  $\text{Fe}^{59}$  in adjacent bone marrow);
- 4) The stores;
- 5) Radiation due to scattering (not illustrated).

tional difficulties arise due to accompanying changes in the distribution of the red cell mass and in associated variations in the ratio of peripheral venous to total body hematocrits.

The *in vivo*  $\text{Fe}^{59}$  counts during the first few hours of the experiment (after correction for the blood radioactivity) can be separated into an extravascular labile component, and into a combination of erythropoietic and storage components (tissue component). Such a decomposition which is applicable to all four groups of models presupposes knowledge of what fraction of the activity leaves the system through each of the two initial pools (plasma and extravascular labile pool), respectively (see Appendix B). Thus, the activity due to the calculated extravascular labile component is different for each assumed mode of exit, that is, for each of the four groups of models (Appendices A and B). Fig. 4 shows decomposition of the experimental *in vivo* data (after correction for blood background), into the extravascular and tissue components calculated for the



mode of exit of iron through either only the first or the second pool respectively (groups I and II), and demonstrates how the two extravascular components differ from each other by a constant factor (constant ordinate distance on semi-logarithmic paper) (see below). These decompositions have been done for data

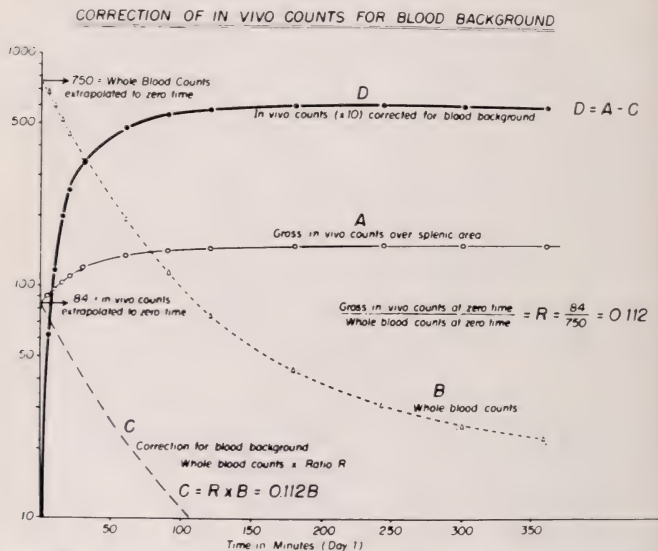


Fig. 3. Illustration of the method used in correcting the gross in vivo counting rates over various body sites (curve A) for the corresponding blood background. The correction curve C is obtained by multiplying the values of the ordinates of curve B (counting rates/ml of whole blood at time  $t$ ) by the ratio

$$R = \frac{\text{gross in vivo counting rate for organ studied, extrapolated to zero time}}{\text{whole blood in vitro counting rate, extrapolated to zero time}}$$

The heavy upper graph D represents the corrected in vivo counting rates (multiplied by a factor of 10), which have been obtained by subtracting the calculated corrections C from the corresponding gross in vivo counting rates A.

obtained over liver, spleen, sacrum, knee, thigh and calf of various patients and the extravascular component has been found universally (for all extreme models (Appendix A)). The observation of the universal distribution of the extravascular labile pool suggests its identification with iron in the interstitial fluid (5, 6, 9). It should be noted that the theoretical tracer behavior values in the second pool as calculated on the basis of an initial two-pool system, do not differ significantly from approximations based upon an initial three-pool system,



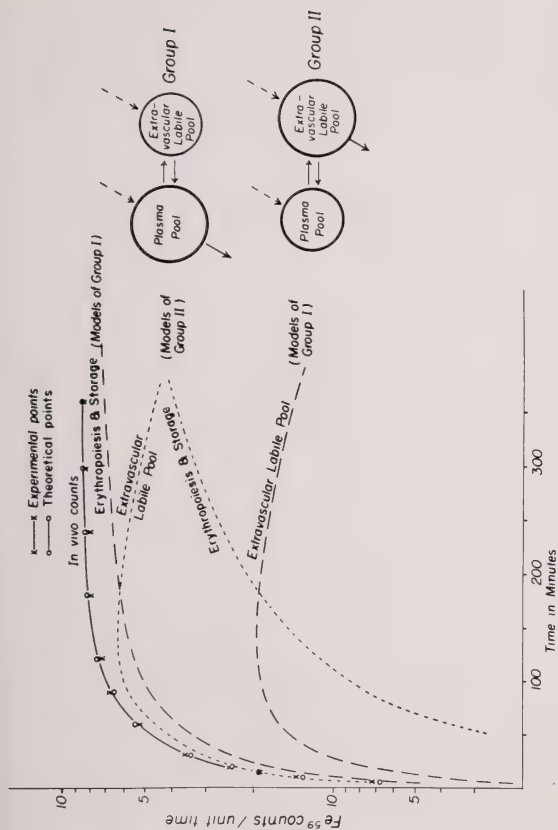


FIG. 4. Analysis of splenic in vivo  $Fe^{59}$  activity based on the assumption of two different groups of models (group I and group II respectively). Separation of the net in vivo radioactivity (corrected for the blood background) over the splenic area into the activity derived from the extravascular labile pool and that derived from the tissue component (erythropoiesis and storage) during the first six hours of the experiment.

during the first four to six hours of observations (i.e., as long as the two approximations to the experimental plasma data are equally satisfactory, which is usually true during the first six hours) (Figs. 5 and 7 in ref. 5 and Table I in ref. 7).

The amount of radioactivity in the extravascular labile pool, as seen by the counter, can be expressed for any given model as a fraction of the initial in vivo blood  $\text{Fe}^{59}$  background. For convenience this fraction will be referred to as "observed normalized in vivo counts" due to the extravascular labile pool. (This fraction will be different for each of the four model-groups considered.) This must be distinguished from the "calculated normalized in vivo counts"

TABLE I  
*Ratios of Maximum "Observed" to "Calculated" \* in vivo*  
*Activity due to the Extravascular Labile Pool*  
Note four extreme values (**boldface**)

Model Group Assumed	Spleen Area										Adjacent Ends of Tibia and Femur			Calf
I	1.2	1.6	<b>7.7</b>	1.2	1.0	2.5	1.7	1.4	1.6	<b>4.5</b>	.9	1.5	1.7	1.1
	1.7	.9	.9	.9	1.2	.9	1.5	.7	1.2		.7	.9	1.7	.8
	1.1	1.3	.9	1.1	1.7	3.3	1.1	2.1	.8		.7	<b>4.7</b>	.5	1.2
II	.9	1.3	.7	1.0	.6	1.9	1.5	1.2	1.8	.6	1.8	.8	1.4	.5
	1.4	1.4	1.0	.8	1.0	1.5	2.1	.5	1.1		.6	1.0	1.8	.4
	1.3	.8	.7	.9	1.3	2.0	2.4	1.7	1.6		.4	1.0	.7	.9
III	1.2	1.5	2.6	1.3	1.0	2.5	1.5	1.4	1.7	1.1	.9	1.5	1.7	.9
	1.6	.7	1.0	.7	1.0	.9	2.1	.6	1.1		.7	.9	1.7	.8
	1.1	1.2	.9	1.0	1.7	3.3	2.4	1.9	1.2		.6	1.8	.6	<b>.2</b>
IV	1.0	1.4	.8	.9	.6	1.9	1.5	1.2	1.8	.8	1.8	.8	1.4	.5
	1.5	.8	1.0	.7	1.0	1.5	2.1	.5	1.2		.7	1.0	1.8	.4
	1.3	.8	.7	.9	1.3	2.0	2.4	1.8	1.5		.4	1.1	.6	.9

\* See text for the definition of "observed" and "calculated."

which represent the activity in the total extravascular pool calculated for each respective model-group directly from plasma  $\text{Fe}^{59}$  disappearance data, and divided by the total initial blood radioactivity which equals the total initial dose administered. If the observed and the calculated normalized in vivo counts are equal, this implies that the same fraction of the total plasma and of the total extravascular pool is seen by the counter. Therefore, if we assume that the decomposition of the early counts over the splenic (or other considered) area into its plasma, extravascular, and tissue components is representative of the total relative activities in the respective pools, the ratio of the maximum "observed" to "calculated" normalized in vivo counts due to the extravascular labile pool should be approximately equal to one. Fig. 5 depicts the four different groups of models utilized in this analysis (see Appendix B). Numerical values of this ratio were calculated on the basis of two-pool analysis for each of these model

groups for 30 experimental subjects (Table I). Three of the four extreme values of the ratio (7.7, 4.5 and 4.7) which are inconsistent with the above assumption of iron pool distribution fall into the first group of models. If this assumption is correct then models of group I may be invalid. The ratios for other model groups, on these subjects, were much closer to one. (Note that now eight two-pool models, namely Ia, Ib, Ic, Id, IIb, IIc, IVb and IVd may be invalid).

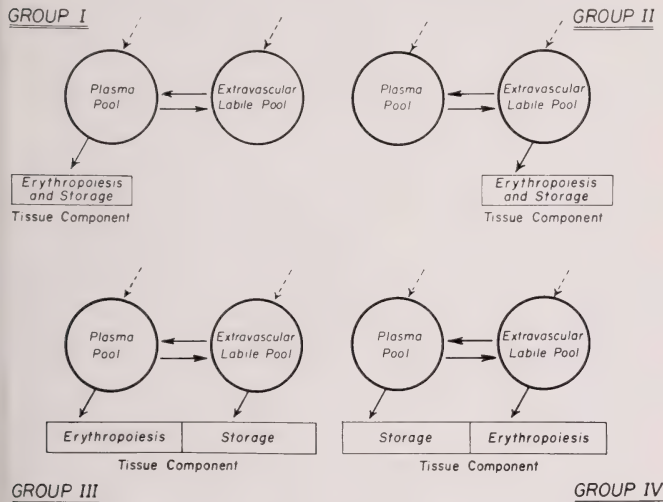


FIG. 5. Schematic representation of four groups of models of a two-pool system, differentiated by the mode of exit of iron:

- Group I: all iron leaves plasma pool;
- Group II: all iron leaves extravascular labile pool;
- Group III: iron used for immediate hemoglobin synthesis leaves plasma pool, iron going to stores leaves extravascular labile pool;
- Group IV: iron going to stores leaves plasma pool, iron used for immediate hemoglobin synthesis leaves extravascular labile pool.

In vivo radioiron studies over prolonged periods of time give further information about the intermediary pathways of iron. The corrected in vivo data on patients with high red cell uptake usually can be approximated after a time lag of one-half to three days by a single exponential function. Surface radioactivity measurements over both the splenic and knee areas of a hematologically normal subject with 95% red cell uptake are given in Fig. 6. The disappearance rates over both sites are approximately the same, thus suggesting essentially a single major source of radioactivity. Such an in vivo disappearance rate is closely reflected in the uptake of  $\text{Fe}^{59}$  in the red blood cells (Fig. 7). This uptake, after

a time lag comparable to the in vivo time lag, can be approximated by a constant, which is equal to the maximum uptake, multiplied by one minus an exponential function whose half time of disappearance is about the same as that of the exponential component of the in vivo radioactivity. Figs. 8 and 9 represent similar data on two subjects with reduced red cell uptakes. The in vivo

IN VIVO RADIOACTIVITY OVER SPLENIC AND KNEE AREAS  
IN A NORMAL SUBJECT WITH 95% RED CELL UPTAKE

(Counts Corrected for Blood Background)

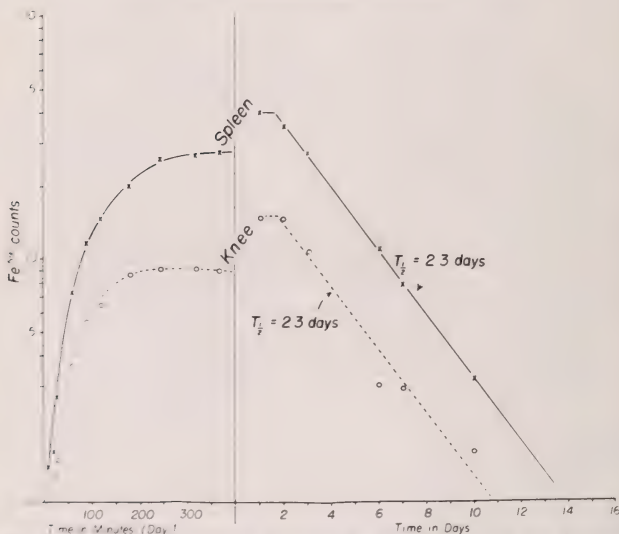


Fig. 6. Net in vivo radioactivity (corrected for blood background) over the splenic area and over the knee of a normal subject with 95% red cell uptake.

The identical slopes ( $T_{1/2} = 2.3$  days) of the  $\text{Fe}^{59}$  in vivo disappearance curves (after a time delay of about two days) indicate essentially the same relative iron turnover rates in the two areas.

counts over spleen and liver sites can be decomposed into two distinct components: a constant, which may be attributed to storage, and a single exponential function. In Figure 9 the plasma disappearance data are approximated by a sum of three exponential functions. The third exponential function,  $ae^{-\lambda_3 t}$ , becomes predominant after the second or third day in plasma as well as in the other compartments of the preerythropoietic phase of iron metabolism (3, 4, 5, 6). This third plasma exponential function is approximately the same as the exponen-

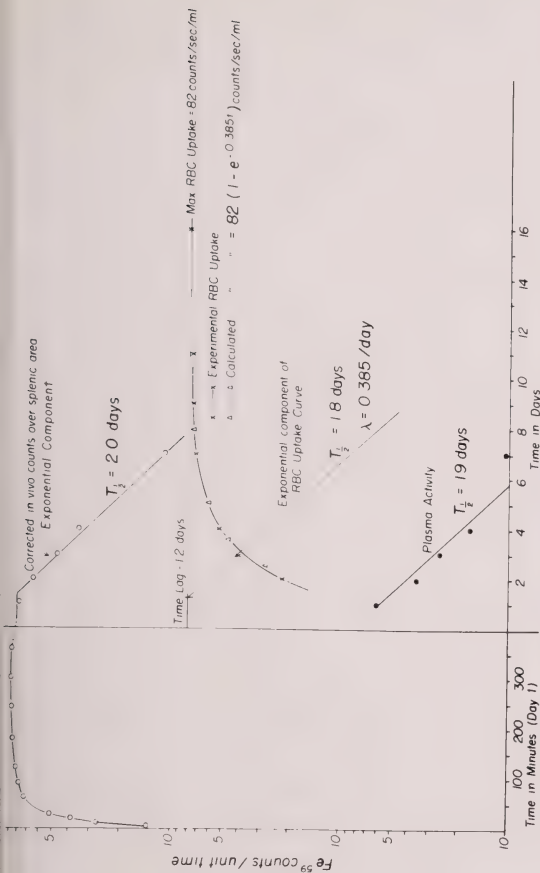


FIG. 7.  $\text{Fe}^{59}$  studies on a patient with secondary polycythemia with 100% red cell uptake. The top curve (O-O-O) represents the in vivo splenic area radioactivity corrected for the blood background. The middle curve (X-X) represents the red cell uptake data in counts/sec/2cc and is approximated by the theoretical curve ( $\Delta-\Delta$ ):  $82(1 - e^{-0.385t})$  with a time delay of 1.2 days. The negative of the exponential component of this curve appears as a straight line on the semi log paper with  $\lambda = 0.385/\text{day}$  and  $T_{1/2} = 1.8 \text{ days}$ . The lowest curve (•-•-•) represents plasma activity in the same patient after the first day. It should be noted that arbitrary ordinates are used for each of the three sets of data. Note almost identical slopes of the in vivo disappearance curve, the exponential component of the RBC uptake after a time lag of about 1.2 days, and the plasma disappearance data after the first day.

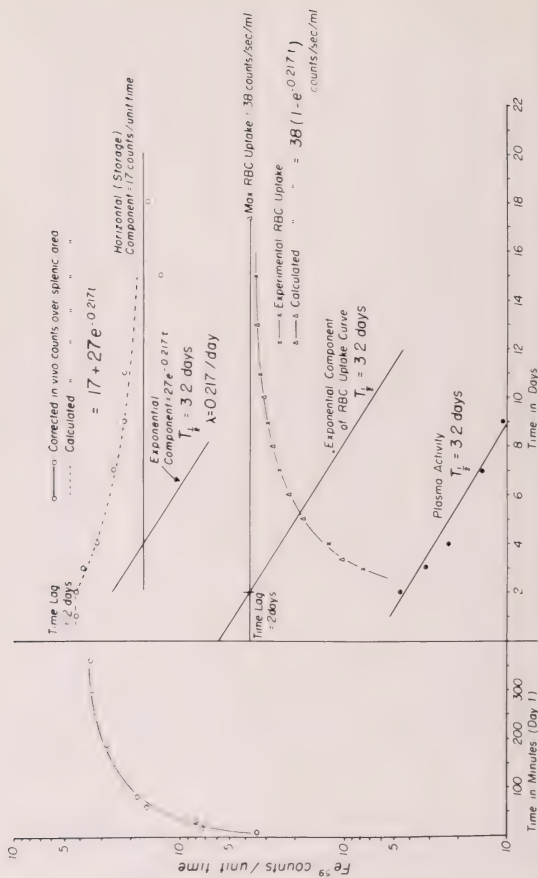


FIG. 8.  $\text{Fe}^{59}$  studies on a subject with 65% red cell uptake. The top curve ( $\circ$ — $\circ$ ) represents the in vivo splenic area radioactivity, corrected for the blood background. This curve is expressed as a sum of two components: a constant component representing stored  $\text{Fe}^{59}$  and an exponential component.

The middle and lowest curves are as per Fig. 7. Note the identical slopes of the exponential components of the in vivo disappearance and RBC uptake curves, and of the plasma disappearance (after day 2).

tial components of the in vivo data. The consistent appearance of the same exponential function in in vivo data, in red cell uptake and in the dominant component of later plasma radioiron data suggests a link among these aspects of iron metabolism. This interrelation forms a basis for the formulation of the general models of iron metabolism (5, 6).

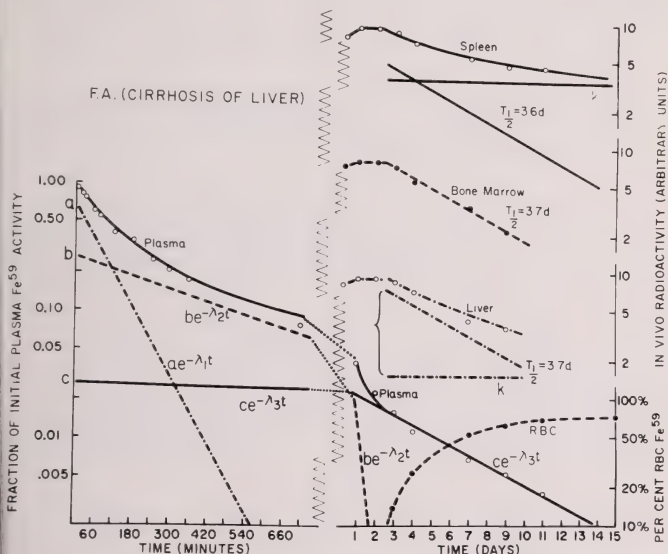


FIG. 9. Subject F.A. (Cirrhosis of Liver): Experimental  $Fe^{59}$  plasma disappearance, red cell uptake and surface radioactivity data.

The plasma  $Fe^{59}$  data are shown decomposed into a sum of three exponential functions. The body surface radioactivity over the liver and spleen sites are decomposed into a constant shown as a horizontal line,  $k$ , and into an exponential component. Over the marrow only an exponential component is present.

It is apparent that the analysis of the metabolic behavior of  $Fe^{59}$  beyond the first six hours cannot be limited to the assumption of an initial two-pool system. A more comprehensive model of iron kinetics is required to interpret adequately the extended (3 days and longer)  $Fe^{59}$  data (2, 5, 6, 7, 10). When hemolysis is present, radioiron from the hemolyzed red cells is recycled through tissue, plasma and red cells. The resulting  $Fe^{59}$  data may not be amenable to the above two- or three-pool analysis. Further refinements, then, may be necessary.

The experimental results discussed in this paper together with the decomposition into three components of the extended plasma  $Fe^{59}$  disappearance data,



serve as a basis for the formulation of a multiple-pool model of normal iron kinetics. Insofar as most of the data considered permit several interpretations (for instance the multiplicity of modes of exit and entry of the initial pool systems) the resulting model will not be unique. The number of such alternative models can, however, be reduced by introducing certain clinical, as well as purely physiological considerations. It is the subject of the other papers of this series (3, 4, 5, 6, 7), to develop such models and to demonstrate the methods of calculation of the respective pool constants and physiological parameters.

#### SUMMARY

Analysis of experimental ferrokinetic data suggesting an interrelationship among radioiron behavior as detected in plasma disappearance, red cell uptake and body surface scanning is presented.

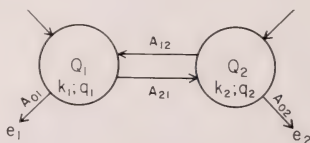


FIG. 10. Schematic representation of a general reduced two-pool system.

$Q_i$  designates both the  $i^{\text{th}}$  pool and the amount of metabolite in the  $i^{\text{th}}$  pool.

$q_i$  represents the amount of radioisotope in the  $i^{\text{th}}$  pool.

$k_i$  is the relative rate; i.e., the fraction of the total metabolite (or of the radioisotope) in the  $i^{\text{th}}$  pool which leaves the  $i^{\text{th}}$  pool per unit time.

$A_j$  is the interchange constant which is defined as that fraction of the total substance leaving the  $i^{\text{th}}$  pool, which leaves the  $i^{\text{th}}$  pool for the  $j^{\text{th}}$  pool. In particular  $A_{oi}$  is such fraction leaving the system as a whole.  $e_i$  represents the fraction of the initial radioactivity,  $q_{oi}$ , which eventually leaves the system via the pool  $Q_i$ .

#### APPENDIX A

This paper concerns itself primarily with the analysis of experimental in vivo scanning data over various body sites. Such data reflect the tracer behavior in plasma both during the first 6 to 8 hours of the experiment and after 2 to 3 days. The plasma  $\text{Fe}^{59}$  disappearance data for two and more days can be best approximated by a sum of three exponential functions (Fig. 7 and Table I in ref. 5) corresponding to a three-pool precerythropoietic system. The general assumptions (such as instantaneous mixing) underlying multiple-pool systems and methods for calculating the constants for one-, two- and three-pool models are discussed in ref. 3, 4 and 5, and are numerically illustrated for several three-pool systems in ref. 7.

The plasma  $\text{Fe}^{59}$  disappearance data for the first 48 hours can usually be equally well approximated by a sum of either three or two (Fig. 5 and 7, and Table I in ref. 5) exponential functions. In general a sum of two exponential functions corresponds to a two-pool system (Figs. 10 and 11). In iron metabolism the two pool precerythropoietic system (Fig. 12) consists of the plasma iron and

the extravascular labile iron pools. The pool constants (such as  $Q_2$  and transfer rates) cannot be determined from the approximating equation  $q_1 = \alpha_1 e^{-\lambda_1 t} + \alpha_2 e^{-\lambda_2 t}$  and plasma iron,  $Q_1$ , alone. Two additional conditions or constraints must be imposed upon the system (2, 3). This can be done in terms of the modes of exit and entry.

Figure 1 represents sixteen such extreme models. The plasma  $\text{Fe}^{59}$  disappearance data on subject S. H. (5) can be analyzed in terms of the above sixteen

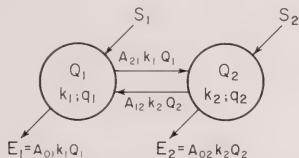


FIG. 11. Schematic representation of a general reduced two-pool system. Here the actual amounts of the metabolite transferred from one pool to another, or leaving the system as a whole, are indicated by annotated arrows (For the exact meaning of the notation see legend of Fig. 10). For instance  $A_{21} k_1 Q_1$  is the amount of metabolite transferred from the pool  $Q_1$  to pool  $Q_2$  per unit time.  $S_i$  and  $E_i$  are the amounts of the metabolite which enter and leave the system as a whole per unit time through the  $i^{\text{th}}$  pool  $Q_i$ .

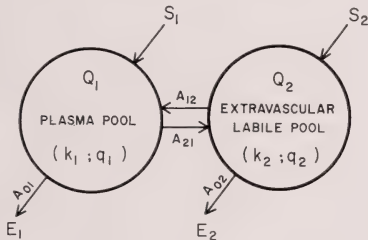


FIG. 12. Schematic representation of a two-pool system consisting of a plasma iron pool and a second pool (extravascular labile iron pool).

models as follows (3):

$$q_1 = 4050e^{-0.00856t} + 700e^{-0.00274t},$$

here  $q_0 = 4050 + 700 = 4750$  and the normalized equation becomes

$$q_1/q_0 = 0.853e^{-0.00856t} + 0.147e^{-0.00274t},$$

and

$$a_1 = 0.853$$

$$a_2 = 0.147$$

$$\lambda_1 = 0.00856$$

$$\lambda_2 = 0.00274.$$

Following the scheme given in ref. 3 we can solve for  $k_1$ ,  $k_2$  and  $A_{12}A_{21}$  as follows:

$$\begin{aligned}
 k_1 + k_2 &= \lambda_1 + \lambda_2 = 0.01130 \\
 k_1 - k_2 &= (a_1 - a_2)(\lambda_1 - \lambda_2) = (0.706)(0.00582) = 0.00411 \\
 \therefore k_1 &= 0.00770 \\
 k_2 &= 0.00360
 \end{aligned}$$

$$(1 - A_{12}A_{21}) = \frac{\lambda_1\lambda_2}{k_1k_2} = \frac{0.0000234544}{0.00002772} = 0.8461,$$

and finally,

$$A_{12}A_{21} = 0.1539.$$

In addition, the maximum RBC uptake,  $U$ , and plasma iron,  $Q_1$ , on patient S. H. are

$$\begin{aligned}
 u &= 0.759 \quad (75.9\%) \\
 Q_1 &= 2.51 \text{ mg.}
 \end{aligned}$$

Substituting these values directly into Table IV of ref. 3 we get Table II.

## APPENDIX B

The gross in vivo counting rate over the splenic or other sites during the first few hours of an experiment is due (1) to the circulating blood activity which is proportional to the activity in the plasma iron pool, (2) to the activity in the extravascular labile pool, and (3) to the activity in the tissue component. The gross counting rate can be corrected for the circulating activity (or blood background) as described in the text. It then remains to decompose the corrected in vivo curve into its components: one proportional to the simultaneous activity in the extravascular labile pool, i.e., to  $q_2(t) = q_0 e^{(-\lambda_2 t)} - e^{(-\lambda_1 t)}$  (3), the other proportional to the accumulating activity in the tissue component. (Such a decomposition presupposed anatomically similar localizations (as detected by the probe) of erythropoietic and storage pools.) Activity in the tissue component depends upon the mode of exit of the initial two-pool system: the accumulated activity,  $q(T.C.;t)^*$ , in the tissue component (assuming no significant loss from this pool during the first six hours) is given by the equation

$$\begin{aligned}
 q(T.C.;t) &= A_{01}k_1 \int_0^t q_1(t)dt + A_{02}k_2 \int_0^t q_2(t)dt \\
 q(T.C.;t) &= A_{01}k_1 q_0 \int_0^t (a_1 e^{-\lambda_1 t} + a_2 e^{-\lambda_2 t})dt + A_{02}k_2 q_0 \int_0^t e^{(-\lambda_2 t)} - e^{(-\lambda_1 t)} dt \text{ or} \\
 q(T.C.;t) &= A_{01}k_1 q_0 \frac{a_1 \lambda_2 + a_2 \lambda_1}{\lambda_1 - \lambda_2} \left[ 1 - \frac{a_1 \lambda_2}{a_1 \lambda_2 + a_2 \lambda_1} e^{-\lambda_1 t} - \frac{a_2 \lambda_1}{a_1 \lambda_2 + a_2 \lambda_1} e^{-\lambda_2 t} \right] \\
 &\quad + A_{02}k_2 q_0 \frac{e^{(-\lambda_1 t)} - e^{(-\lambda_2 t)}}{\lambda_1 - \lambda_2} \left[ 1 + \frac{\lambda_2}{\lambda_1 - \lambda_2} e^{-\lambda_1 t} - \frac{\lambda_1}{\lambda_1 - \lambda_2} e^{-\lambda_2 t} \right] \quad (3).
 \end{aligned}$$

(Hence the corrected in vivo data over an initial period of 6-8 hours consist

\*  $q(T.C.;t)$  = Total cumulative activity at time  $t$ .

of  $L_1 q_2(t) + L_2 q(T.C.;t)$ , (where  $L_1$  and  $L_2$  are constants) and can be expressed in terms of a constant and two exponential functions  $e^{-\lambda_1 t}$  and  $e^{-\lambda_2 t}$  with constant coefficients.)

When all exit is through pool I, i.e., plasma (corresponding to models of group I),  $A_{02} = 0$  and  $q(T.C.;t)$  is proportional to the first term on the right of the above equation. When all exit is through pool II, i.e., extravascular labile pool (models of group II),  $q(T.C.;t)$  is proportional to the second term. If a given corrected in vivo curve can be decomposed into an extravascular labile component  $L_1 q_2(t)$ , and a component  $q(T.C.;t)$ , based on the assumption of the first group of models (all exit through plasma), it can also be similarly analyzed for all other pool models. To prove this it suffices to show that the two quantities in brackets (of the above equation) differ from each other by an amount proportional to the activity in the extravascular pool:\*

$$\begin{aligned} & \left[ \quad \right]_1 - \left[ \quad \right]_2 = \\ &= \left[ 1 - \frac{a_1 \lambda_2}{a_1 \lambda_2 + a_2 \lambda_1} e^{-\lambda_1 t} - \frac{a_2 \lambda_1}{a_1 \lambda_2 + a_2 \lambda_1} e^{-\lambda_2 t} \right] - \left[ 1 + \frac{\lambda_2}{\lambda_1 - \lambda_2} e^{-\lambda_1 t} \right. \\ & \quad \left. - \frac{\lambda_1}{\lambda_1 - \lambda_2} e^{-\lambda_2 t} \right] \\ &= \left[ \frac{a_1 \lambda_2}{a_1 \lambda_2 + a_2 \lambda_1} + \frac{\lambda_2}{\lambda_1 - \lambda_2} \right] e^{-\lambda_1 t} + \left[ -\frac{a_2 \lambda_1}{a_1 \lambda_2 + a_2 \lambda_1} + \frac{\lambda_1}{\lambda_1 - \lambda_2} \right] e^{-\lambda_2 t} \\ &= \frac{a_2 \lambda_1 \lambda_2 + a_1 \lambda_1 \lambda_2}{(a_1 \lambda_2 + a_2 \lambda_1)(\lambda_1 - \lambda_2)} [e^{-\lambda_2 t} - e^{-\lambda_1 t}] \\ &= \frac{\lambda_1 \lambda_2}{(a_1 \lambda_2 + a_2 \lambda_1)(\lambda_1 - \lambda_2)} [e^{-\lambda_2 t} - e^{-\lambda_1 t}]. \end{aligned}$$

Therefore, if the fraction of the total activity which leaves through either of the two pools (Fig. 1), is known, the in vivo counts due to the extravascular pool or to the tissue pool respectively, can be calculated for each of the sixteen models, or rather each of the four groups of models assumed.

\* For, assume that the corrected in vivo data are decomposed into two components  $L_{1I} q_2(t)$  and  $L_{2I} q_I(T.C.;t)$ , where  $q_I(T.C.;t)$  corresponds to the "tissue pool" for models of group I, and therefore consists of only the first term of the general equation for  $q(T.C.;t)$ . Then for any other pool model  $Y$ , the ratio of the cumulative activities

$$R(Y) = \frac{A_{01}(Y) k_1 q_0 \int_0^t q_{1I} dt}{A_{02}(Y) k_2 q_0 \int_0^t q_{2I} dt}$$

can be calculated, and  $L_{2I} q_I(T.C.;t)$  can be changed into  $L_{2Y} q_Y(T.C.;t)$  by subtracting an appropriate constant  $M$  times the difference of the two brackets ( $[ ]_1 - [ ]_2$ ) of the general expression for  $q(T.C.;t)$ . The linear combination  $L_{1I} q_2(t) + L_{2I} q_I(T.C.;t)$  becomes now  $L_{1I} q_2(t) + L_{2Y} q_Y(T.C.;t) - M([ ]_1 - [ ]_2) + M([ ]_1 - [ ]_2) = L_{1Y} q_{2Y}(t) + L_{2Y} q_Y(T.C.;t)$ .

TABLE II

*Calculations of physiological parameters of iron metabolism on patient S.H. for sixteen extreme two-pool models*

Here  $A_{12}A_{21} = 0.1539$ ,  $k_1 = 0.00770$  min.,  $k_2 = 0.00390$  min.,  $U = 0.759$  and  $Q_1 = 2.51$  mg. In column 1 factor  $F$  is calculated from the above values. Column two gives values of the extravascular labile iron pool in mg; column three gives the effective iron turnover for the two-pool system ( $R_e$ ) in mg/day; column four gives the effective fractional red cell iron uptake ( $U_e$ ) as distinct from the fractional radioiron uptake ( $U'$ ); and column five gives the effective red cell iron turnover ( $U'R_e$ ) in mg/day.

Factor ( $F$ )	Models whose values for $Q_2$ are obtained by multiplying the corresponding factor $F$ by $Q_2 = (k_1/k_2)Q_1 = 5.469$ mg	Models whose values for $R_e$ are obtained by multiplying the corresponding factor $F$ by $R_e = (1 - A_{12}A_{21})k_2Q_1 = 0.01615$ mg/min = 24.55 mg/day	Models whose values for $U_e$ are obtained by multiplying the corresponding factor $F$ by $U = 0.759$	Models whose values for $U'R_e$ are obtained by multiplying the corresponding factor $F$ by $R_eU = (1 - A_{12}A_{21})k_2Q_1U = 0.01241$ mg/min = 17.87 mg/day
1	$Q_2$ (Ib); IIa; IIId; IVc) = 5.37 mg	$R_e$ (Ia, b, c, d; IIa; IIId; IVa) = 23.5 mg/day	$U'$ (Ia, b, c, d; IIa; b, c, d; IIIa; IVa) = 0.759	$U'R_e$ (Ia, b, c, d; IIa; d; IVa) = 17.9 mg/day
$A_{12}A_{21} = 0.1539$	$Q_2$ (Ia) = 0.826 mg	$R_e$ (IIb) = 153 mg/day		$U'R_e$ (IIb; IVb) = 116 mg/day
$\frac{1}{A_{12}A_{21}} = 6.499$	$Q_2$ (IIb) = 34.9 mg			
$1 - U'(1 - A_{12}A_{21}) = 0.3578$	$Q_2$ (Ic; IIIa) = 1.92 mg			
$\frac{1}{1 - U'(1 - A_{12}A_{21})} = 2.795$	$Q_2$ (IIId) = 15.0 mg	$R_e$ (IIId) = 65.8 mg/day		$U'R_e$ (IIId) = 50.0 mg/day
$\frac{1}{1 - U'(1 - A_{12}A_{21})} = 2.325$	$Q_2$ (IIIb) = 12.5 mg	$R_e$ (IIIb) = 54.7 mg/day		
$A_{12}A_{21}$			$U_e$ (IIIb) = 0.326	
$\frac{A_{12}A_{21}}{1 - U'(1 - A_{12}A_{21})} = 0.1304$				
$1 - U'(1 - A_{12}A_{21}) = 0.7961$	$Q_2$ (Id; IVa) = 4.27 mg	$R_e$ (IIc) = 29.6 mg/day	$U_e$ (IVb) = 0.953	$U'R_e$ (IIc; IVc) = 22.5 mg/day
$\frac{1}{A_{12}A_{21} + U'(1 - A_{12}A_{21})} = 1.256$	$Q_2$ (IIc) = 6.74 mg			
$\frac{1}{A_{12}A_{21} + U'(1 - A_{12}A_{21})} = 5.173$	$Q_2$ (IVb) = 27.8 mg	$R_e$ (IVb) = 122 mg/day		$U'R_e$ (IVd) = 92.5 mg/day

$\frac{[A_{12}A_{21} + U(1 - A_{12}A_{21})]^2}{A_{12}A_{21}} = 4.118$	$Q_2 \text{ (IVd)} = 22.1 \text{ mg}$		
$\frac{[1 - U(1 - A_{12}A_{21})]^2}{A_{12}A_{21}} = 0.8319$	$Q_2 \text{ (IIIc)} = 4.47 \text{ mg}$		
$\frac{1 - U(1 - A_{12}A_{21})}{1 - U^2(1 - A_{12}A_{21})} = 0.6980$			$U_c \text{ (IIIId)} = 0.530$
$\frac{A_{12}A_{21} + (1 - U)^2(1 - A_{12}A_{21})}{A_{12}A_{21}} = 1.319$		$R_c \text{ (IIIc)} = 31.1 \text{ mg/day}$	
$\frac{A_{12}A_{21}}{A_{12}A_{21} + (1 - U)^2(1 - A_{12}A_{21})} = 0.7580$			$U_c \text{ (IIIc)} = 0.575$
$\frac{1 - U^2(1 - A_{12}A_{21})}{1 - U(1 - A_{12}A_{21})} = 1.433$		$R_c \text{ (IIId)} = 33.7 \text{ mg/day}$	
$\frac{1 - (1 - U)^2(1 - A_{12}A_{21})}{A_{12}A_{21} + U(1 - A_{12}A_{21})} = 1.194$		$R_c \text{ (IVc)} = 28.1 \text{ mg/day}$	
$\frac{1}{1 - (1 - U)^2(1 - A_{12}A_{21})} = 1.052$			$U_c \text{ (IVc)} = 0.798$
$\frac{A_{12}A_{21} + U^2(1 - A_{12}A_{21})}{A_{12}A_{21}} = 4.167$		$R_c \text{ (IVd)} = 98.1 \text{ mg/day}$	
$\frac{A_{12}A_{21} + U(1 - A_{12}A_{21})}{A_{12}A_{21} + U^2(1 - A_{12}A_{21})} = 1.241$			$U_c \text{ (IVd)} = 0.942$

It is convenient to express the *in vivo* counting rate over a given area, due to either extravascular or to tissue component, as a fraction of the circulating activity over the same area extrapolated to zero time. Thus expressed, the activity is independent of the amount of radioisotope injected. The values in Table I are the ratios of the *in vivo* activity at a given time due to the extravascular component expressed as a fraction of the extrapolated initial activity, divided by the corresponding calculated total activity injected into the plasma. The actual calculations in each case refer to the maximum activity in the extravascular pool.

This time of maximum activity can be obtained by differentiating  $q_2(t) = c(e^{-\lambda_2 t} - e^{-\lambda_1 t})$  with respect to  $t$  and setting equal to zero:

$$\begin{aligned} c(-\lambda_2 e^{-\lambda_2 t} + \lambda_1 e^{-\lambda_1 t}) &= 0 \\ \lambda_2 e^{-\lambda_2 t} &= \lambda_1 e^{-\lambda_1 t} \\ \log \lambda_2 - \lambda_2 t &= \log \lambda_1 - \lambda_1 t \\ \therefore t_{q_2 \max} &= \frac{\log_e \lambda_1 - \log_e \lambda_2}{\lambda_1 - \lambda_2} \end{aligned}$$

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## Studies in Iron Kinetics

### III. Formulation of the Models of Iron Metabolism\*

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In studying the kinetics of iron metabolism, it is desirable to formulate mathematical analogues or models of the dynamic processes under consideration. The characteristics of such models are of necessity determined by the type of experimental data available (in this case isotopic tracer data), as well as by the particular orientation of the investigation. In iron metabolism the bulk of iron in a normal individual is derived from exogenous sources, and intestinal absorption of iron may appear to be a logical starting point for the formulation of models describing dynamic processes of iron distribution. However, analogues which are based upon plasma  $\text{Fe}^{59}$  disappearance data are intimately concerned with the iron pathways emanating from plasma to red cells, to storage, etc., and, therefore, plasma is used as the focal point for the models of iron metabolism.

Initially a single exponential function  $q(t) = q_0 e^{-kt}$  † was found to give an adequate approximation to the plasma  $\text{Fe}^{59}$  disappearance data both in normal subjects and in patients having various hematological disorders. Such an approximation implies a single plasma iron pool  $Q$  (Fig. 1) (4) with iron leaving and entering at a constant absolute rate  $kQ$  per unit time. A constant fraction  $U$  of  $kQ$  is utilized directly for hemoglobin synthesis and the remaining fraction  $1 - U$  goes to iron stores. As a clinical tool this simple one-pool model may be useful for diagnostic purposes. The half-time of plasma  $\text{Fe}^{59}$  disappearance ( $T_{1/2} = 0.693/k$ ) is obtained from the approximating equation  $q(t) = q_0 e^{-kt}$  (Fig. 2), or directly from its straight-line graph on semi-logarithmic paper. The various blood disorders may then be grouped according to the numerical values of the corresponding half-times of  $\text{Fe}^{59}$  disappearance from plasma (Fig. 3) (4, 5). When total plasma iron, maximum red cell uptake of  $\text{Fe}^{59}$  and red cell mass are known, it is possible to calculate the plasma iron turnover, red cell iron turnover, and rate of renewal of hemoglobin, on the basis of this one-pool concept (4).

As mentioned above, in formulating models or analogues of physiological processes, special attention must be given to the type of experimental data available for mathematical analysis. Linear combinations of exponential functions

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† For definitions and notations used in this paper see "Studies in Iron Kinetics: I," (1) and "Multiple Pool Analysis in Tracer Studies of Metabolic Kinetics: I and II" (2, 3).

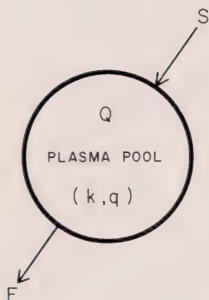


FIG. 1.

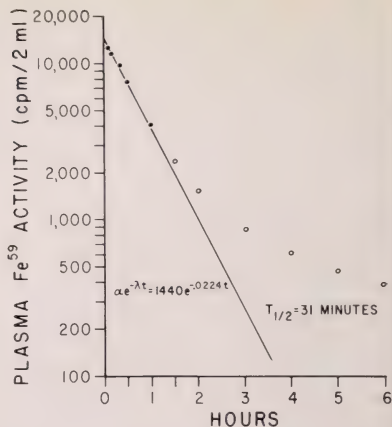


FIG. 2.

FIG. 1. Schematic representation of a one-pool model of the preerythropoietic phase of iron metabolism.

FIG. 2. C.W. (Polycythemia vera): approximation of plasma  $\text{Fe}^{59}$  disappearance data during the first hour of the experiment by a single exponential function.

... Experimental data during the first hour of the study.

— Approximation to the above data by

$$q = \alpha e^{-\lambda t} = 1440 e^{-0.0224 t} \quad (t \text{ in min})$$

ooo Experimental data after the first hour.

(For similar data and results see references 1 and 12.)

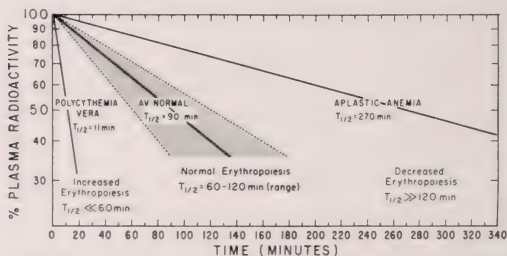


FIG. 3. Comparison of plasma radioiron disappearances. The shaded area represents the normal range with an average  $T_{1/2} = 90$  min. Note the very rapid half time of disappearance in the presence of hyperactive erythropoiesis (such as in polycythemia vera), and the markedly prolonged  $T_{1/2}$  when red cell production is diminished or absent (such as in aplastic anemia).

$(\alpha_1 e^{-\lambda_1 t} + \alpha_2 e^{-\lambda_2 t} + \alpha_3 e^{-\lambda_3 t} + \cdots + \alpha_n e^{-\lambda_n t})$  are particularly suited for approximations to the tracer data in the studies of various steady-state metabolic systems. In general, sums of  $n$  exponential functions describe the mathematical behavior of a tracer in  $n$ -pool models (2, 3), assuming (1) instantaneous mixing, and (2) constant relative rates of transfer (or first order reactions), or (3) a state of dynamic equilibrium. Theoretically, a set of tracer data can be equally well approximated by any one of a large variety of families of curves (not necessarily exponential functions). As a rule, however, such representations are both very complicated, and lack the uniform logical foundation and simplicity inherent in the use of exponential functions in the characterization of the above multiple-pool models. This becomes especially obvious, for example, if one tries to represent data which can be adequately approximated by a single exponential function, by a power series  $\alpha_0 + \alpha_1 t + \alpha_2 t^2 + \cdots + \alpha_n t^n$  or, for that matter, by any other type of function.

The one-pool plasma model appeared satisfactory as long as the experimental tracer data could be adequately characterized by a single exponential function. With the development of better detecting devices, this representation proved no longer satisfactory (7, 8, 9). A sum of two exponential functions,  $q(t) = \alpha_1 e^{-\lambda_1 t} + \alpha_2 e^{-\lambda_2 t}$  became necessary to approximate the plasma  $\text{Fe}^{59}$  disappearance data for the first six hours after injection of the tracer (Fig. 4) (7). Such a representation corresponds to a two-pool model (Fig. 5) where the first, or accessible, pool is plasma, and the "second" pool is denoted as extravascular labile pool. This pool has been identified with iron in the interstitial fluid (1, 6, 10).

From the clinical point of view, the two-pool model has certain disadvantages: besides losing the simplicity of a one-pool model, the two-pool system can no longer be characterized by the half-time of disappearance of  $\text{Fe}^{59}$  from plasma (7). The new equivalent parameter is that of the effective rate,  $R_e$ , of iron disappearance from the two-pool system as a whole, and not from plasma alone (2, 7). The two-pool model gives better approximation to the experimental data and leads to an evaluation of total red cell iron turnover and per cent hemoglobin renewal, which in most blood disorders with altered erythropoiesis yields values more compatible with present concepts of hemoglobin metabolism (1, 7).

Huff's one pool model (4), and the early two-pool models (7) form isolated systems in the sense that they are studied independently of other aspects of iron metabolism. No interrelationships between plasma radioiron data and in vivo data was either sought for or alluded to. For example, no attempts were made to analyze the initial phase of in vivo data (up to 6 or 12 hours) thereby failing to bring out its underlying physiological significance.

With extensive information derived from in vivo counting over multiple body sites, yielding characteristic patterns in normal subjects and in various hematologic disorders (11), the isolated one- or two-pool system becomes too unsophisticated, and more comprehensive models of iron kinetics are necessary. Here again the mathematical analysis starts with that of plasma  $\text{Fe}^{59}$  disappearance data for the first six hours after injection, which is approximated by a sum of two exponential functions and hence is represented by a two-pool system. All

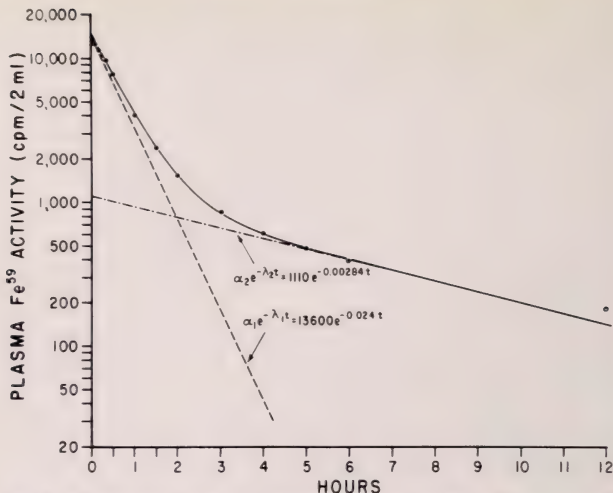


FIG. 4. C.W. Polycythemia vera: approximation of plasma  $\text{Fe}^{59}$  disappearance data during the first six hours of the experiment by a sum of two exponential functions.

... Experimental data during the first six hours of the study.

— Approximation to the above data by  $q_1 = \alpha_1 e^{-\lambda_1 t} + \alpha_2 e^{-\lambda_2 t} = 13,600 e^{-0.024 t} + 1110 e^{-0.00284 t}$  in min.

- - - Exponential function:  $\alpha_1 e^{-\lambda_1 t} = 13,600 e^{-0.024 t}$

- - - Exponential function:  $\alpha_2 e^{-\lambda_2 t} = 1110 e^{-0.00284 t}$

○ Experimental data after first six hours.

(For similar data and results see references 1 and 12)

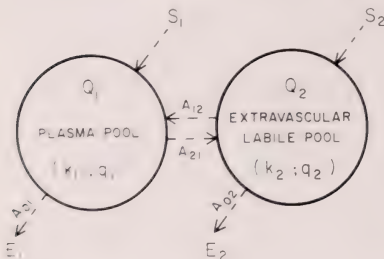


FIG. 5. Schematic representation of a reduced (2) two-pool system, consisting of a plasma iron pool and an extravascular labile iron pool.

$Q_1$  and  $Q_2$  denote the "First Pool" and the "Second Pool" respectively.

constants for defining such a two-pool model (Fig. 5) cannot be obtained directly from the approximating equation describing the tracer behavior in plasma (Fig. 4) (2, 7). Additional information is required to determine the exact modes of exit and entry of iron from and into this two-pool system (2, 6; Appendix A).

In the following formulation of the multiple-pool model of the kinetics of iron metabolism, this two-pool component is represented as a single unit which, in view of its physiological function, is designated Transport Sub-System (Figs. 6 and 11) and which, as a whole, interchanges with erythropoietic and storage pools and possibly other reservoirs. The exact modes of the connections of the two individual pools with the remainder of the system (exit and entry) remain unspecified for the present. If the plasma  $\text{Fe}^{59}$  disappearance data are considered over the period of two to three days after injection, they can be empirically decomposed into a sum of three exponential functions,  $q(t) = \alpha_1 e^{-\lambda_1 t} + \alpha_2 e^{-\lambda_2 t} + \alpha_3 e^{-\lambda_3 t}$  (Fig. 7), thus indicating the existence of a third iron pool (Fig. 8) (2) which significantly influences the behavior of the tracer in plasma during this extended time interval. In normal subjects when the plasma  $\text{Fe}^{59}$  disappearance data are approximated by a sum of three exponential functions, the functions containing the two larger negative exponents do not, as a rule, differ considerably

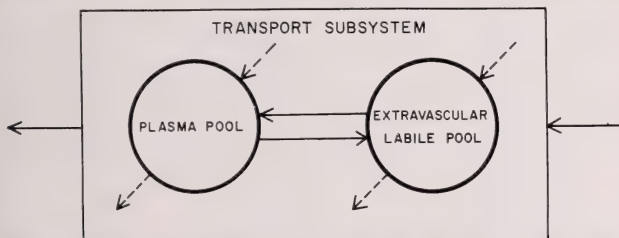


FIG. 6. Schematic representation of the iron transport sub-system. The fact that the exact modes of exit and entry are not known is indicated by the broken arrows.

from the exponential functions  $\alpha_1 e^{-\lambda_1 t}$  and  $\alpha_2 e^{-\lambda_2 t}$  describing the behavior of the two-pool system during the first six hour period. Furthermore, the numerical values of the pool constants, which are calculated on the basis of an initial two-pool system remain essentially the same when they are recalculated on the assumption of certain three-pool systems (12). It will be convenient to denote this third pool as the "marrow iron transit" pool, though different pathways for the transport iron are mathematically admissible (Fig. 8). One such alternative is discussed below. In the third component,  $\alpha_3 e^{-\lambda_3 t}$ , of a decomposition into a sum of three exponential functions, however, both the coefficient,  $\alpha_3$ , and the constant of the exponent,  $-\lambda_3$ , are of a lower order of magnitude than  $\alpha_1$ ,  $\alpha_2$  and  $-\lambda_1$ ,  $-\lambda_2$ , respectively. The half-time of disappearance,  $0.693/\lambda_3$ , of this third component is measured in terms of days, instead of minutes and hours, as for  $0.693/\lambda_1$  and  $0.693/\lambda_2$ , respectively. In addition, the exponential function  $\alpha_3 e^{-\lambda_3 t}$ , which forms the predominant component of the tracer equation for plasma after the first day of the experiment, is approximately the same (except for the magnitude of the coefficient) as the predominant function describing the in vivo radioactivity over various body surface sites during the same period

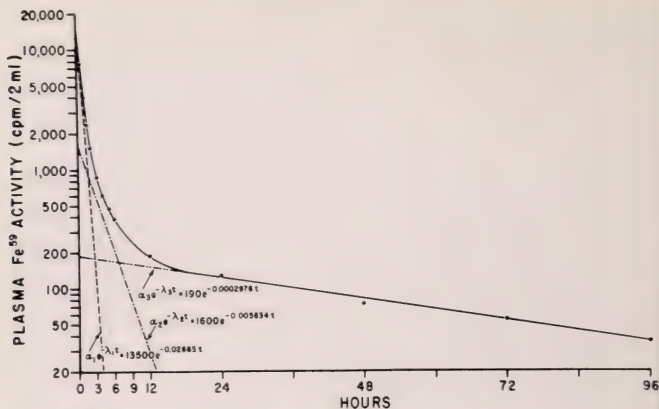


FIG. 7. C.W. Polycythemia vera: approximation of plasma  $\text{Fe}^{59}$  disappearance data during the first four days of the experiment approximated by a sum of three exponential functions.

... Experimental data during the first four days of the study.

— Approximation to above data by  $q = \alpha_1 e^{-\lambda_1 t} + \alpha_2 e^{-\lambda_2 t} + \alpha_3 e^{-\lambda_3 t} = 13,500 e^{-0.026654 t} + 1,600 e^{-0.005634 t} + 190 e^{-0.0002976 t}$

(Note that  $t$  in equation is expressed in minutes)

--- Exponential function:  $\alpha_1 e^{-\lambda_1 t} = 13,500 e^{-0.026654 t}$

- - - Exponential function:  $\alpha_2 e^{-\lambda_2 t} = 1,600 e^{-0.005634 t}$

- - - Exponential function:  $\alpha_3 e^{-\lambda_3 t} = 190 e^{-0.0002976 t}$

(For similar data and results see references 1 and 12)

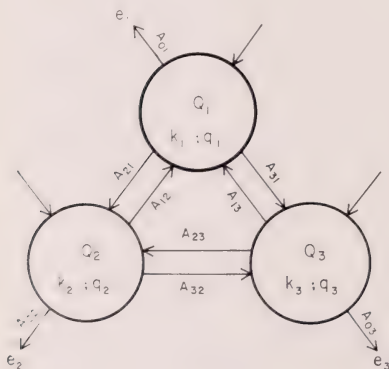


FIG. 8. Schematic representation of a general three-pool system. For definitions see reference 1 (Figs. 1 and 2) and reference 2.

except for a time lag of about one day (6). This can be interpreted to signify that the tracer behavior, both in plasma and over such body surface sites, is affected not only by the initial two-pool system, i.e., the transport subsystem, but also by the same third pool, the "marrow iron transit" pool (Fig. 9). If the plasma  $\text{Fe}^{59}$  disappearance data are determined beyond two or three weeks, further exponential components may be indicated. The half-time of disappearance of such a fourth exponential function,  $\alpha_4 e^{-\lambda_4 t}$ , would be, again, of a lower

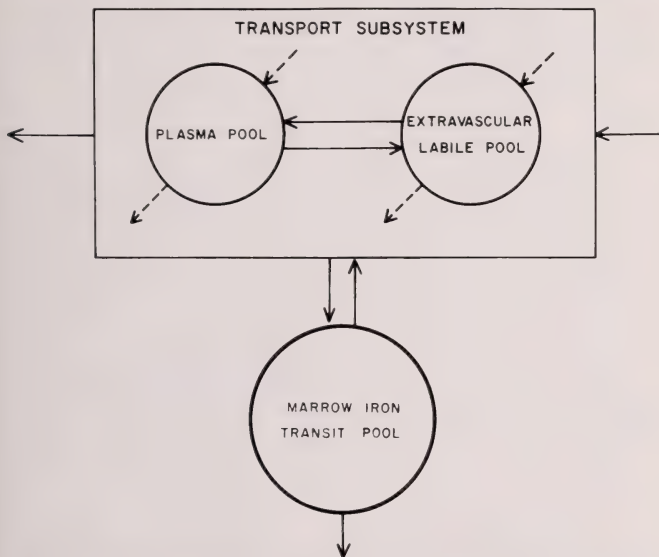


FIG. 9. Schematic representation of the initial reduced three-pool system of iron metabolism, consisting of the transport iron subsystem and the marrow iron transit pool.

order of magnitude than those of any of the preceding ones, and the coefficient would be so small that the addition (or subtraction) of this function to the plasma disappearance data during the first few hours or days would fall within the limits of experimental errors. The numerical constants of such a fourth component (as well as of any possible additional components) have not been determined because at this stage the levels of activity in plasma are very low, and because this component may be masked by the presence of post-erythropoietic radioactivity such as due to normal ineffective erythropoiesis (13).

Four exponential components in the plasma  $\text{Fe}^{59}$  disappearance curve would indicate a four-pool system (2). The fourth pool would represent a storage pool,



which is labeled with radioiron leaving the transport subsystem but which is not initially destined for hemoglobin synthesis (Fig. 10). The existence of such a storage pool (which may be spatially distributed over widely separated areas) should be verifiable by means of *in vivo* counting (6). This should be particularly demonstrable in cases of diminished red cell uptake (aplastic anemia, refractory anemias, etc.) with no appreciable hemolysis. Thus, in Figure 11, one notes the *in vivo* counts over the liver and splenic areas of a subject with no hemolysis and only 73 per cent red cell incorporation of radioiron. These data can be resolved into two distinct components, an exponential function, and a constant which may be attributed to localization of radioiron in storage pools.

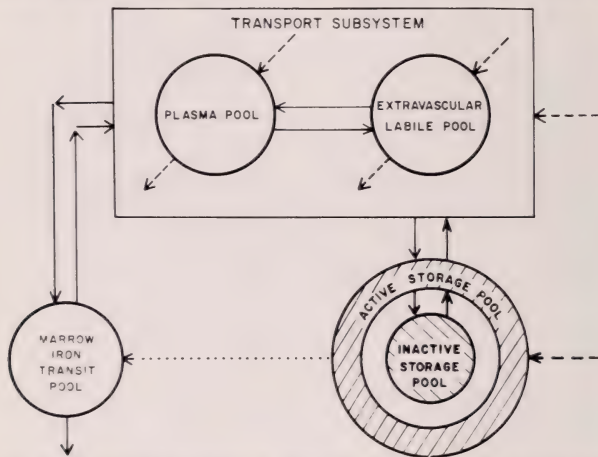


FIG. 10. Schematic representation of iron interchange between the transport subsystem and the marrow iron transit pool and storage pools respectively.

In most normal patients considered, the red cell uptake averaged over 90 per cent. A 90 per cent red cell uptake, however, allows for a storage localization of about 10 per cent of the initially injected tracer dose. Such storage activity can be completely masked by over-correction for the highly radioactive blood background. The correction for circulating blood background, as previously described (6) is based upon the assumption that the ratio of the fraction of the total plasma activity initially seen by the *in vivo* counter, to the fraction of the total red cell activity subsequently detected, is equal to the ratio of the fraction of total plasma to the fraction of total red cell mass contained in the sampling tube for measuring the activity of venous whole blood. Such an assumption is equivalent to postulating equal peripheral venous and local tissue or organ hematocrits. This is not generally true, since the tissue hematocrits over splenic and other

areas may differ significantly from peripheral venous hematocrits. Therefore, in normal subjects or patients with high hematocrits, after the first 24 hours, when the circulating plasma activity is replaced by red cell activity, relatively less blood background may be seen by the in vivo counter than is calculated on the assumption of equal peripheral venous and local hematocrits. The correction for the true circulating blood background after the first experimental day may then have to be reduced to 90 or even 80 per cent of the calculated value, to

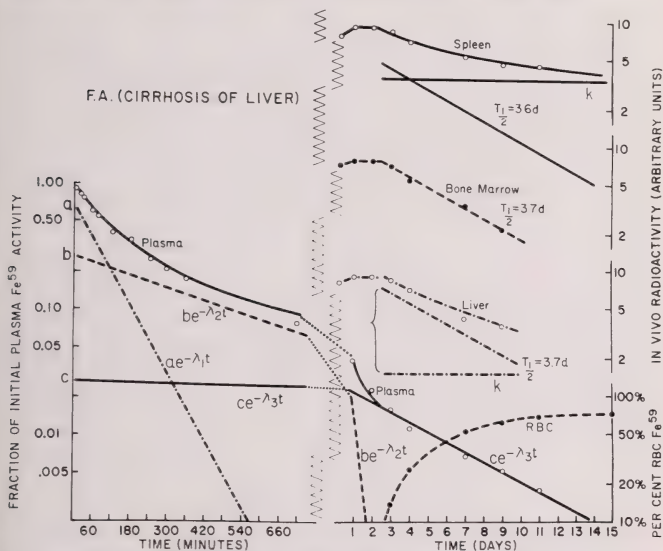


FIG. 11. Experimental plasma  $Fe^{59}$  disappearance data, red cell uptake data and surface radioactivity data on the subject F.A. (cirrhosis of liver).

account for the difference between the venous and local hematocrits. Such corrections, when applied to the gross in vivo counting data of the above subjects, lead to in vivo curves exhibiting significant horizontal (constant) components of radioactivity. Since such corrections differ from individual to individual and cannot be determined a priori, other methods for quantitating and localizing storage deposits must be devised.

Another phenomenon illustrated in Figure 11, is that of a time delay between the initial appearance of radioiron at various organ sites and its subsequent reappearance in circulating red cells.\* This delay is essentially the time lag which

\* Note that erythropoietic activity is normally detected over all organ sites (6).

is defined as the interval between the disappearance of iron from the reduced three-pool system and its reappearance in red cells, and can be represented by a series of unidirectionally connected pools, i.e., a sequence of irreversible reactions, comprising the different stages of erythrocyte maturation, and the concomitant elaboration of hemoglobin (6, 14, 15). In figure 12, this sequence is denoted by the "erythron maturation" pool. The *in vivo* data representing the combined activity of the "marrow iron transit" and "erythron maturation" pools, behave,

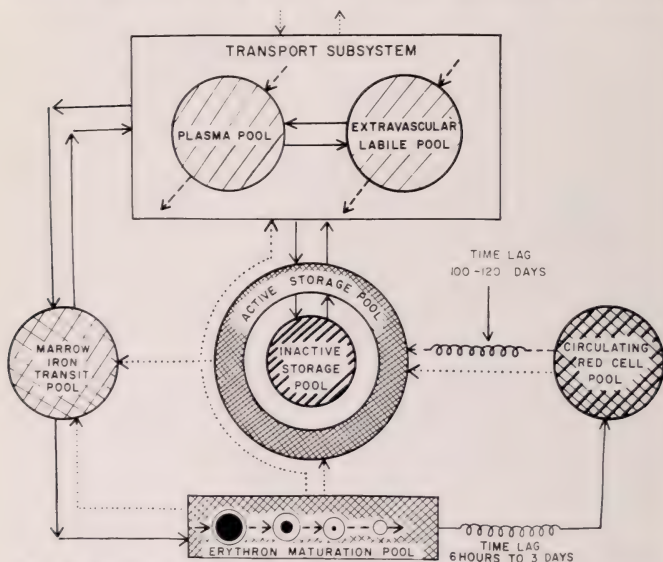


FIG. 12. Schematic representation of multiple-pool models of iron kinetics in normal subjects.

after most of the iron leaves the transport subsystem, approximately as a single pool with a delay of six hours to three days. The appearance of radioiron in the circulating red cell pool proceeds as if iron were transferred directly from the "marrow iron transit" pool, i.e., with no intermediary pools, though again with the same time lag. The process of senescence of circulating red cells can also be considered as an infinite sequence of unidirectionally connected pools (or irreversible reactions) manifesting itself as another time lag of 100-120 days, at the end of which time the hemoglobin iron is released by the hemolysis of the effete cells (14). It is commonly accepted that iron from catabolized erythrocytes is preferentially reutilized for hemoglobin synthesis (16, 17). Additional observa-

tions (17, 18) suggest that iron derived from hemoglobin breakdown may enter a metabolically active storage pool from which it is mobilized in random fashion for the maintenance of the steady state of the transport subsystem.

This completes the proposed model of iron kinetics in normal human subjects, describing the major movements and distribution of iron as deduced from the available  $\text{Fe}^{59}$  data. These massive movements of  $\text{Fe}^{59}$  may, however, mask additional minor interchanges between various pools. Thus, even in normal subjects, there is physiological ineffective erythropoiesis (13), and the transport of iron from these hemolyzed cells may not coincide with the major iron movements (solid lines Fig. 12). Such auxiliary pathways are indicated in Figure 12 by dotted lines. Direct uptake of plasma  $\text{Fe}^{59}$  by circulating reticulocytes, as well as utilization of radioiron for myoglobin, cytochromes, and other enzymes, is not detectable in  $\text{Fe}^{59}$  plasma disappearance, red cell uptake, and in vivo counting data, and have been disregarded in the formulation of the above multiple-pool model. It should be noted that theoretically the order of appearance of radioiron in the marrow iron transit and erythron maturation pools cannot be detected by in vivo measurements alone. The in vivo counters will register the same activity if, instead of the above model, one postulates the direct incorporation of iron into the developing red cell precursors (with the corresponding six hours to three days time lag) with the subsequent release of the mature cells into a red cell reservoir, from which red cell iron is finally transferred (with a half-time disappearance of one to three days) partly into the peripheral circulation, and partly after a metabolic change back to the transport subsystems. In theory, the order of the above pools should be detectable in the plasma  $\text{Fe}^{59}$  disappearance data. In practice, however, the difference in activities due to an immediate return of  $\text{Fe}^{59}$  from the "marrow iron transit" pool, and a delayed return due to the recycling after a time lag, may not be large enough to be distinguished from the usual experimental errors. The possible "recycling of siderocyte iron" (19, 20, 21) falls within the confines both of the proposed model (Fig. 12) and of the above hypothetical alternative.

In considering a model of iron kinetics, it should be borne in mind that various pools may not be anatomically distinct and need not be localized in contiguous areas or single organs. Thus plasma and red cell pools both occupy the same vascular spaces; the extravascular labile pool (or interstitial fluid) overlaps the storage and erythron maturation pools; and finally, the marrow iron transit pool may not be spatially distinct from either storage or erythron maturation pools.

Some speculations about the chemical and physiological nature of marrow iron transit pool can be gleaned from the literature. Pollycove et al. (22, 23, 24) suggest that the "labile erythropoietic pool" \* may be exchangeable iron on the surface of immature erythrons. They observed that in man and in dogs most of the marrow radioiron remains in non-heme form thirty-six and eight hours after injection, respectively. Specifically, the "miscible storage iron" (ferritin) in mar-

\* The "labile erythropoietic pool" in their three-pool analysis is mathematically equivalent to the marrow iron transit pool in Model IIIA (1, 12).

row is distinguished from the above mentioned pool by its lack of lability. This is at variance with the findings of Noyes and Finch (25) who found in rabbits that 80% of the marrow radioiron is in heme during the period of 15 minutes to four hours after injection. Zail and co-workers (26) described (in man and rats) ferritin formation in maturing red cells only when "more iron enters the cell than can be immediately utilized for hemoglobin synthesis," and observed that the remaining ferritin is removed from the red cell prior to its release into the circulation. They suggest that this removed ferritin-iron may contribute to a labile marrow pool which may feed back into the plasma. Mazur's studies on rat marrow and reticulocytes, and human reticulocytes (27), however, present evidence for the initial iron incorporation into ferritin prior to its incorporation into heme. According to Bessis and Breton-Gorius (28) the ferritin iron in reticulum "nurse cells" moves from reticulum cells to maturing erythrons by the process of "rhopheocytosis," as seen by the electron microscope. To what extent species and strain specificities and methodology account for these different findings is problematical.

Jandl and Katz's experiments (29) give strong indication that the transferrin-iron complex can be reversibly bound to reticulocytes. Upon dissociation of the transferrin-iron complex the protein recirculates; the iron is mainly metabolized in the interior of the cell, although it can also be released back into the circulation. Such surface iron may contribute to the labile erythropoietic pool, as previously postulated by Pollycove et al. (22, 23, 24) for "immature erythrons."

The marrow iron transit pool may encompass some or all of the above forms of iron; conceptually this pool does not require any specific anatomic or chemical delineation. The "erythron maturation pool" is primarily iron irreversibly destined for hemoglobin synthesis and hemoglobin iron within the erythrocyte precursors. This pool can be interpreted as an "infinite" series of maturing heme containing cells comprising (or as irreversible reactions characteristic of) the different stages of erythropoiesis, interposed between the marrow iron transit and the circulating red cell iron pools. In normal erythropoiesis some of the cells produced do not effectively contribute to the circulating red cell pool (normal "ineffective" erythropoiesis) (13, 30, 31, 32). Therefore, the iron in these cells may be extraneous to the erythron maturation pool. Alpen and Cranmore (33) observed radioactive remnants of nucleated erythroid cells and erythrocytes in marrow reticulo-endothelial cells within 24 hours after addition of  $\text{Fe}^{59}$ . From their autoradiographic studies they postulate the presence of a "labile non-plasma (iron) pool" and comment that continued labelling of developing erythrons "may result from a destruction of a certain small number of labelled cells." This suggests that the iron involved in normal ineffective erythropoiesis forms a part of the marrow iron transit pool.

In iron kinetic studies the erythron maturation pool manifests itself as a time lag between the disappearance of radioiron from the "preerythropoietic phase" and its subsequent reappearance in the peripheral blood. This represents the mean time span from irreversible iron fixation in red cell precursors to its release into the circulation in red cells. This time lag is not to be confused with the total



maturation time of red cells which has been estimated to be approximately 3 days (33, 34), and which includes all stages of red cell development.

The multiple-pool system, as represented in Figure 12, is at best a simplified working model of iron kinetics in normal subjects. When a hemolytic component, peripheral hemolysis and or "ineffective erythropoiesis" is present, some or even most of the iron from the circulating red cell pool may return to the rest of the system (active stores or transport subsystem) in a random fashion (dotted lines in Figure 12). Such iron pathways are superimposed upon the usual ones which are due to normal destruction of senescent red cells and which are manifested by a "time lag." Thus, the exact pathway by which iron from destroyed red cells returns to the rest of the system, may vary as a function of both senescence and random hemolytic processes. In certain blood dyscrasias, where normoblasts are present among circulating red cells, some radioiron will be transferred directly from plasma into the circulating red cell pool, bypassing the accepted metabolic sequence. On the other hand, in the present dynamic concept of interchanging systems, one should not differentiate between pools on the basis of their spatial distribution alone; the circulating normoblasts may be considered part of the bone marrow whereby iron is supposedly transferred directly from plasma into "erythron maturation" pool. The apparent bypassing of the "marrow iron transit" pool may actually represent a failure to recognize the iron-bound transferrin molecules enveloping normoblasts as part of the non-contiguous "marrow iron transit" pool. This suggests that models of iron kinetics in various blood dyscrasias may differ significantly from that proposed for normal subjects (Fig. 12).

#### SUMMARY

Models of iron metabolism based upon multiple-pool analysis of ferrokinetic data are presented and discussed.

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## Studies in Iron Kinetics:

### IV. Calculation of Physiological Parameters on the Basis of Multiple-Pool Models\*

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In the preceding papers of this series (1, 2, 3) a set of models for iron kinetics has been developed. In normal subjects, where no significant hemolysis of  $\text{Fe}^{59}$  labelled red cells occurs during the initial few days of the study, the reduced systems for these models consist of only three distinct pools.† A reduced system is defined (1, 4, 5, 6) as that part of a total pool system in which bilateral (though not necessarily direct) interchange exists between any two component pools, and in which, therefore, the tracer behavior in any given pool is a function of the pool constants of all component pools. Thus the tracer distribution functions for each component pool consist of linear combinations of the same set of exponential functions. The three component pools of the above reduced system comprise "plasma," "extravascular labile" and "marrow iron transit" pools (Fig. 1).

In order to determine completely (to calculate all parameters of) such a reduced three-pool system several assumptions must be made: the modes of exit of iron from the two-pool transport subsystem and the entry of iron into the reduced three-pool system must be decided upon (2, 4, 5). It is postulated that no iron enters the system through the marrow iron transit pool and that, therefore, storage iron both leaves and enters by means of the transport subsystem. In this context, the iron destined for hemoglobin synthesis leaves one of the two pools of the transport subsystem and is transferred to the erythron maturation pool (1, 3) via the marrow iron transit pool.

Theoretically any one of the sixteen extreme models (ref. 2, Fig. 1) may be chosen for the transport subsystem. The choice of the model is somewhat simplified by previous exclusion from such considerations of models IIb, IIc, IVb, IVc (2, 7) and probably of all models of group I (2). The remaining models appear in Figure 2. In order to reduce the number of workable models it is arbitrarily assumed that all iron enters through the plasma pool or through the extravascular labile pool but not through both. (This leaves only four models: IIa, IIIa, IIIb and IVa (Fig. 2).) It is further assumed that the marrow iron

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† For a brief discussion of a possible initial four-pool reduced system see references 1 and 3.

transit pool of the reduced three-pool system is interchanging bilaterally with only one of the component pools of the transport subsystem. This allows for only one possible reduced three-pool system corresponding to each of the above four two-pool transport models (Fig. 3).

It is the purpose of this paper 1) to demonstrate how the plasma  $\text{Fe}^{59}$  disappearance data can, in most cases (where sufficient plasma  $\text{Fe}^{59}$  data are available and where no significant hemolysis of  $\text{Fe}^{59}$  tagged cells occurs during the

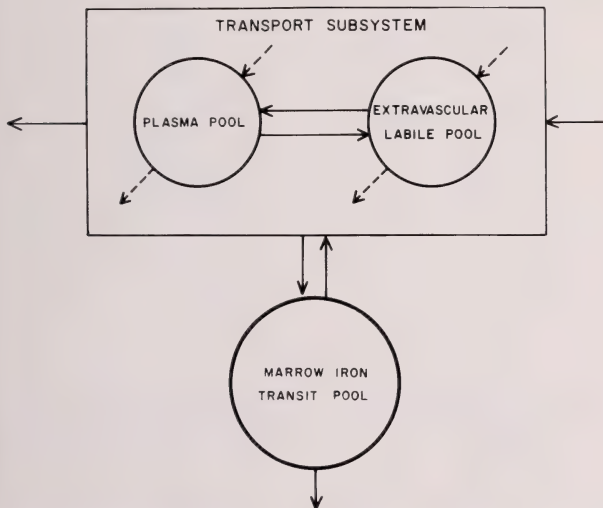


FIG. 1. Schematic representation of the initial three-pool subsystem of iron metabolism in normal subjects. The plasma pool and the extravascular labile pool form the transport subsystem. The broken arrows indicate uncertainty of interconnections (2, 4).

first two days of the experiment) be analyzed in terms of the above four alternative partial models of iron metabolism; 2) to compare the results of such analyses in hematologically normal subjects with those in patients with various blood dyscrasias; and finally, 3) to collate in different subjects the red blood cell iron turnover rates derived from the three-pool analysis with those based on the assumptions of initial two- and one-pool models, respectively.

As an example, the plasma  $\text{Fe}^{59}$  disappearance data will first be approximated by a sum of three exponential functions

$$\frac{q_1}{q_0} = a_1 e^{-\lambda_1 t} + b_1 e^{\lambda_2 t} + c_1 e^{\lambda_3 t}.$$

The pool constants (including such physiologically significant parameters as the size of the marrow iron transit pool and the RBC iron turnover rate) will then be determined in detail for each of the four alternative models in terms of the constants of the approximating equation (i.e.,  $a_1$ ,  $b_1$ ,  $c_1$ ,  $\lambda_1$ ,  $\lambda_2$  and  $\lambda_3$ ),

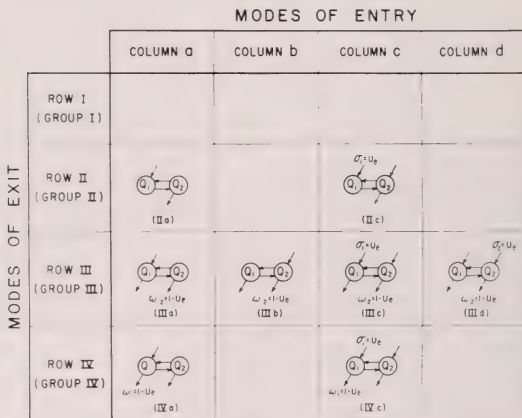


FIG. 2.

*Eight Admissible Two-Pool Models of Iron Transport Subsystem*

- Column a: All entry of iron to the reduced\* three-pool system through plasma pool  $Q_1$ .  
 Column b: All entry of iron to the reduced system through extravascular labile pool  $Q_2$ .  
 Column c: Fraction  $\sigma_1 = U_e^{**}$  of total amount of iron entering the reduced system enters through plasma pool  $Q_1$ ; fraction  $\sigma_2 = 1 - U_e$  enters through extravascular labile pool  $Q_2$ .  
 Column d: Fraction  $\sigma_1 = 1 - U_e$  of total amount of iron entering the reduced system enters through plasma pool  $Q_1$ ; fraction  $\sigma_2 = U_e$  enters through extravascular labile pool  $Q_2$ .  
 Row I: All exit of iron from transport subsystem through plasma pool  $Q_1$ .  
 Row II: All exit of iron from transport subsystem through extravascular labile pool  $Q_2$ .  
 Row III: Fraction  $U_e$  of the total iron leaving the reduced system leaves it though marrow iron transit pool via plasma pool  $Q_1$ ; fraction  $1 - U_e$  leaves the transport subsystem through the extravascular labile pool  $Q_2$ .  
 Row IV: Fraction  $U_e$  of the total iron leaving the reduced system leaves it though marrow iron transit pool via extravascular labile pool  $Q_2$ ; fraction  $1 - U_e$  leaves transport subsystem through plasma pool  $Q_1$ .

\* Here "reduced" system refers always to reduced three-pool system.

\*\*  $U_e$  = effective RBC iron uptake

Amount of iron leaving the reduced three-pool system to be utilized for Hgb. synthesis  
 Total amount of iron leaving the reduced three-pool system

the total plasma iron,  $Q_1$ , and the maximum fractional RBC  $\text{Fe}^{59}$  uptake,  $U$  (5, 6). In addition, the analysis will be extended to include:

(a) A comparison of experimental with calculated (theoretical) RBC  $\text{Fe}^{59}$  uptake data for all models, including a determination of the "time lag" (8) and hence the size of the erythron maturation pool (Fig. 6).

(b) A comparison of experimental with calculated *in vivo* counting data for the initial models IIIa and IIIb (Fig. 3).

The following data obtained from the  $\text{Fe}^{59}$  tracer study of 50 year old female patient, A.M., with an undiagnosed neurologic disorder, will be utilized for

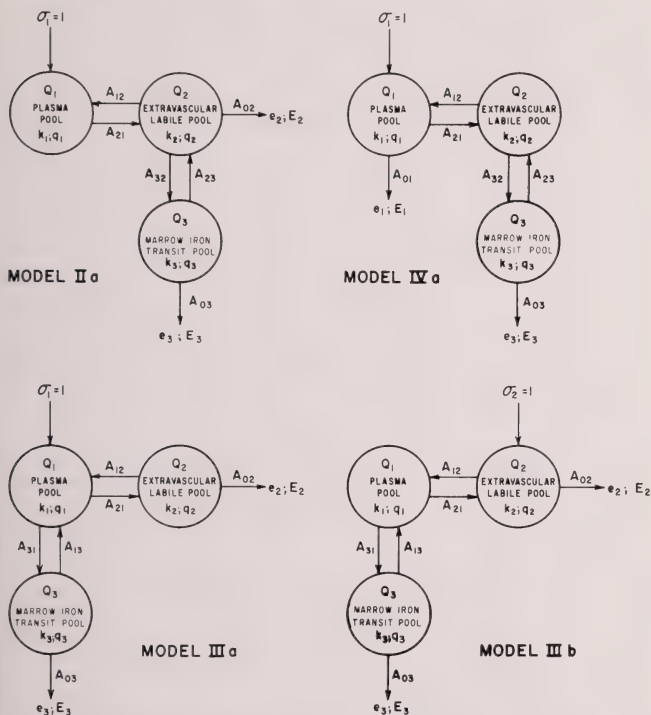


FIG. 3. Schematic representation of the four reduced three-pool subsystems of iron metabolism, corresponding to the two-pool models IIa, IIIa, IIIb and IVa.

analysis in terms of initial reduced three-pool systems. (Each set of  $\text{Fe}^{59}$  data is corrected for a single reference date):

1. Plasma  $\text{Fe}^{59}$  disappearance data (Table I, column 1) in terms of counts per minute per 2 ml of plasma.
2. RBC  $\text{Fe}^{59}$  uptake data (Table II, column 1) in terms of counts per minute per 2 ml of hemolyzed whole blood.

TABLE I  
*Experimental Plasma  $F_{100}$  Disappearance Data on Patient A.M., and Their Approximation by Two Different Sums of Three Exponential Functions*

First approximation: $q' = 3030e^{-0.00030t} + 4200e^{-0.00019t} + 210e^{-0.01019t}$														
Second approximation: $q'' = 710e^{-0.01019t} + 6400e^{-0.00030t} + 325e^{-0.00030t}$														
Column	Sampling time after $F_{100}$ injection	First approximation					Second approximation					Deviations from ex- perimental values as % of theoretical values (column 12)		
		1	2	3	4	5	6	7	8	9	10		11	12
		Experimental values: counts per min. per 2 ml of plasma	210e <sup>-0.01019t</sup> time in min.	Column 1 minus Column 2	4200e <sup>-0.00030t</sup> time in min.	3030e <sup>-0.00030t</sup> time in min.	Theoretical values: counts per 2 + 1 + 5 columns	Deviations from ex- perimental values as % of theoretical values (column 6)	125e <sup>-0.00030t</sup> time in min.	6400e <sup>-0.00030t</sup> time in min.	710e <sup>-0.00030t</sup> time in min.	Theoretical values: counts per 9 + 10 + 11 columns		
0			210		4200	3030	7140		325	6400	710	7435	0.0	
5 min.		7253	210	7043	4137	2940	7281	28	0.3	324	6283	7254	-0.1	
10 "		7141	210	6931	4075	2853	7138	-3	0.0	324	6167	641	7132	-0.1
22 "		6942	209	6733	3931	2654	6794	-148	-2.1	322	5899	567	6788	-2.3
30 "		6573	209	6364	3838	2529	6576	3	0.0	322	5723	523	6568	-0.1
60 "		5850	208	5642	3506	2110	5824	-26	-0.4	318	5124	385	5827	-0.4
90 "		5149	207	4942	3203	1761	5171	22	0.4	315	4585	284	5181	0.7
120 "		4614	205	4409	2927	1470	4602	-12	-0.3	311	4102	209	4622	0.2
180 "		3705	203	3502	2443	1024	3670	-35	-1.0	305	3284	113	3702	-0.1
243 "		2900	201	2699	2021	700	2922	22	0.8	298	2601	60	2959	2.0
318 "		2283	198	2085	1613	445	2256	-27	-1.2	290	1969	28	2287	0.2
358 "		1988	197	1791	1430	350	1977	-11	-0.6	286	1699	26	2011	1.1
640 "		859	187	672	612	64	863	4	0.4	259	597	1	857	-0.2
1430 "	(= 1 d)	221.5	161.2	60.3	56.7	0.5	218.4	-3.1	-1.4	195.2	32.0	227.2	227.2	2.5
2 days		124.3	123.3	1.0	0.7		124.0	-0.3	-0.2	116.4	0	116.4	116.4	-6.8
3 days		69.6	94.5	-24.9			94.5	24.9	26.3	69.7	0	69.7	69.7	0.1
6 days		42.5	42.5	0			42.5	0	0.0					
10 days		14.6	14.7	-0.1			14.7	0.1	0.7					

\* Time in minutes.

3. In vivo  $\text{Fe}^{59}$  activity (corrected for blood background) over a bone marrow area (sacrum) (Table III) (ref. 2, Fig. 3).

4. Total plasma iron ( $Q_1 = 4.27$  mg. Maximum RBC  $\text{Fe}^{59}$  uptake  $U = 0.99$  (expressed as a fraction of total initial dose injected). Total  $\text{Cr}^{51}$  red cell mass = 1695 ml.

The plasma  $\text{Fe}^{59}$  disappearance data (Table I, column 1, and Figure 4) can be approximated by a sum of three exponential functions as follows: a straight line drawn on semilog paper through the three points representing the plasma activity after two, six and ten days, respectively (Figure 4) corresponds to the

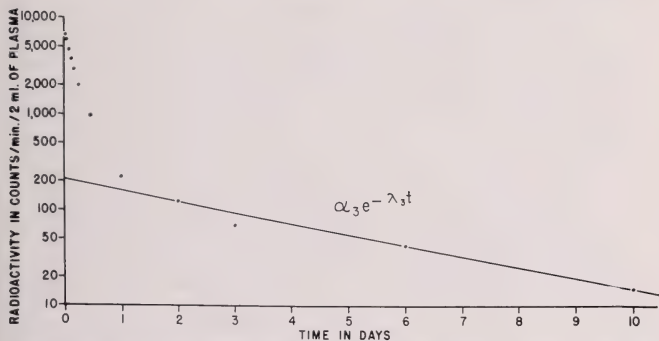


FIG. 4. Approximation of the plasma  $\text{Fe}^{59}$  disappearance data on patient, A.M., by a sum of three exponential functions

$$q_1 = \alpha_1 e^{-\lambda_1 t} + \alpha_2 e^{-\lambda_2 t} + \alpha_3 e^{-\lambda_3 t}.$$

#### Part I:

- • • Experimental plasma  $\text{Fe}^{59}$  disappearance data during the first 10 days of the experiment (Table I, column 1).
- The third exponential component: Approximation of the experimental points after the second day by the exponential function

$$\alpha_3 e^{-\lambda_3 t} = 210e^{-0.0001848t} \quad (t \text{ in minutes}) \quad (\text{Table I, column 2}).$$

exponential function  $210e^{-0.0001848t} = 210e^{-\lambda_3 t}$ , where  $t$  is in minutes. The corresponding value of the function for various sampling times are recorded in Table I, column 2. Column 3 represents the difference between the experimental values and the function  $210e^{-\lambda_3 t}$  at the sampling times, i.e., values of [column 1-column 2]. These values are plotted in Fig. 5 and can, in two additional steps, be approximated by a sum of two exponential functions, namely  $4200e^{-0.00301t} = 4200e^{-\lambda_2 t}$  (Table I, column 4) and  $3030e^{-0.00603t} = 3030e^{-\lambda_1 t}$  (Table I, column 5). The theoretical values expressed by the approximating equation

$$q_1 = 3030e^{-0.00603t} + 4200e^{-0.00301t} + 210e^{-0.0001848t}$$

corresponding to the various sampling times,  $t$ , (i.e., the sums of columns 2, 4



and 5) are given in column 6 of Table I. Finally, the differences between the experimental and theoretical values are given both in terms of counts per minute per 2 ml and as per cent of theoretical values, in columns 7 and 8. To obtain the required constants  $a_1$ ,  $b_1$ , and  $c_1$  (6) the approximating equation must be normalized, i.e.,  $q_1$  and each of the coefficients of the exponential functions must

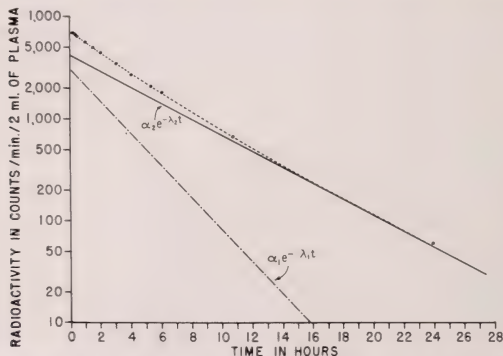


FIG. 5. Approximation of the plasma  $\text{Fe}^{59}$  disappearance data on patient A.M., by a sum of three exponential functions

$$q_1 = \alpha_1 e^{-\lambda_1 t} + \alpha_2 e^{-\lambda_2 t} + \alpha_3 e^{-\lambda_3 t}.$$

Part II:

... Experimental plasma  $\text{Fe}^{59}$  disappearance data minus the corresponding values of the exponential function  $\alpha_3 e^{-\lambda_3 t} = 210e^{-0.0001848t}$  ( $t$  in minutes) during the first day of the experiment (Table I, column 3).

..... Approximation of the plasma  $\text{Fe}^{59}$  disappearance data minus the corresponding values of  $\alpha_3 e^{-\lambda_3 t} = 210e^{-0.0001848t}$ , by the sum of two exponential functions

$$\alpha_1 e^{-\lambda_1 t} + \alpha_2 e^{-\lambda_2 t} = 3030e^{-0.00603t} + 4200e^{-0.00301t}$$

( $t$  in minutes) (Table I, columns 4 + 5).

— The first exponential component  $\alpha_1 e^{-\lambda_1 t} = 3030e^{-0.00603t}$  (Table I, column 5).

- - - The second exponential component  $\alpha_2 e^{-\lambda_2 t} = 4200e^{-0.00301t}$  (Table I, column 4).

be divided by the sum of the coefficients =  $3030 + 4200 + 210 = 7440 =$  theoretical activity  $q_0$  at zero time:

$$q_1 = a_1 e^{-\lambda_1 t} + b_1 e^{-\lambda_2 t} + c_1 e^{-\lambda_3 t}$$

$q_0$

$$= 0.4073e^{-0.00603t} + 0.5645e^{-0.00301t} + 0.0282e^{-0.0001848t}$$

Hence, the constants of the approximating tracer equation of the plasma become:

$$a_1 = 0.4073; \quad b_1 = 0.5645; \quad c_1 = 0.0282$$

$$\lambda_1 = 0.00603; \quad \lambda_2 = 0.00301; \quad \lambda_3 = 0.0001848.$$

Note that  $a_1$ ,  $b_1$  and  $c_1$  are absolute numbers, i.e., indices, while  $\lambda$ 's are numbers per unit time, in this particular case, per minute. In the following,  $Q_1$  will always refer to the plasma pool,  $Q_2$  to the extravascular labile pool and  $Q_3$  to the marrow iron transit pool.

The actual calculations follow the scheme indicated in reference 6. The three symmetric functions of  $\lambda_i$ 's become

$$s_1 = \lambda_1 + \lambda_2 + \lambda_3 = 0.0092248$$

$$s_2 = \lambda_1\lambda_2 + \lambda_2\lambda_3 + \lambda_3\lambda_1 = 0.0000198209$$

$$s_3 = \lambda_1\lambda_2\lambda_3 = 0.00335418 \times 10^{-6}$$

The tracer distribution systems (5) for models IIIa and IIIb (Fig. 3) correspond to case 2 of a "three-pool system with the accessible pool in the center" (ref. 6, Figs. 2 and 4), with  $e_2$  = fraction of tracer leaving through pool  $Q_2$  (i.e., through extravascular labile pool) =  $1 - U = 0.01$ ;  $e_1 = 0$ ;  $e_3 = U = 0.99$ . Then:

$$k_2 + k_3 = X_1 = a_1(\lambda_2 + \lambda_3) + b_1(\lambda_1 + \lambda_3) + c_1(\lambda_1 + \lambda_2) = 0.00506442.$$

$$k_2k_3 = X_2 = a_1\lambda_2\lambda_3 + b_1\lambda_1\lambda_3 + c_1\lambda_1\lambda_2 = 1.367445 \times 10^{-6}.$$

From the above the constants  $k_2$  and  $k_3$  can be determined as usual:

$$k_2, k_3 = \frac{k_2 + k_3 \pm \sqrt{(k_2 + k_3)^2 - 4k_2k_3}}{2} = \frac{0.00506442 \pm 0.00449196}{2}$$

Comparing the three-pool analysis with the results obtained with two-pool models, it follows as a rule that  $k_2 > k_3$ . Hence

$$k_2 = 0.00477824$$

$$k_3 = 0.00028618$$

$$k_1 = 0.00416038 = s_1 - (k_2 + k_3).$$

The above relative rates  $k_i$ , as well as the following interchange constants, refer to rates of models of group III, and whenever necessary will be designated as  $k_i(\text{III})$ , etc. Furthermore,

$$A_{12}A_{21}(\text{III}) = \frac{s_3 - k_2s_2 + k_2^2(k_1 + k_3)}{k_1k_2(k_2 - k_3)} = 0.113856$$

$$A_{13}A_{31}(\text{III}) = \frac{s_2k_3 - s_3 - k_3^2(k_1 + k_2)}{k_1k_3(k_2 - k_3)} = 0.296560$$

$$A_{21} = A_{12}A_{21} + \frac{e_2s_3}{k_1k_2k_3} = 0.119752$$

$$A_{12} = \frac{A_{12}A_{21}}{A_{21}} = 0.950766$$

$$A_{02} = 1 - A_{12} = 0.049234$$

$$A_{31} = 1 - A_{21} = 0.880248$$

$$A_{03} = \frac{e_3 s_3}{k_1 k_2 k_3 A_{31}} = 0.663094$$

$$A_{13} = 1 - A_{03} = 0.336906 = \frac{A_{13} A_{31}}{A_{31}}$$

The constants  $Q_2$ ,  $Q_3$ ,  $E_2$  and  $E_3$  can be determined for models IIIa and IIIb by imposing the appropriate equilibrium conditions as described in reference 6:

$$Q_3(\text{III}) = \frac{k_1}{k_3} A_{31} Q_1 = 54.6 \text{ mg (marrow iron transit pool)}$$

$$E_3(\text{III}) = \text{RBC iron turnover} = k_3 A_{03} Q_3 \times 1440 \text{ min./day} = 14.9 \text{ mg./day}$$

$$\text{Red cell renewal} = \frac{E_3 \times 100}{\text{RBC volume}} = \frac{14.9 \times 100}{1695} = 0.88\% \text{ per day.}$$

Model IIIa:  $s_1 = 1$ ;  $s_2 = s_3 = 0$ .

$$Q_2(\text{IIIa}) = \frac{k_1}{k_2} A_{21} Q_1 = 0.445 \text{ mg (extravascular labile pool)}$$

$$E_2(\text{IIIa}) = k_2 A_{02} Q_2 \times 1440 = 0.1507 \text{ mg./day (iron to storage (1))}$$

Model IIIb:  $s_2 = 1$ ;  $s_1 = s_3 = 0$

$$Q_2(\text{IIIb}) = \frac{k_1}{k_2} \left( \frac{1}{s_2} - \frac{A_{13} A_{31}}{A_{12}} \right) Q_1 = 2.75 \text{ mg}$$

$$E_2(\text{IIIb}) = k_2 A_{02} Q_2 \times 1440 = 0.931 \text{ mg./day}$$

Models IIa and IVa (Fig. 3) correspond (except for the interchange of pools  $Q_1$  and  $Q_2$  and the consequent alteration in numbering of pool constants) to case 3 and case 2 of a "three-pool system in series with the accessible pool  $Q_1$  at one end," respectively (ref. 6, Figs. 5, 7, 8):

$$k_3(\text{II,IV}) = \frac{X_2 s_1 - X_1 X_2 - s_3}{X_1 s_1 + X_2 - X_1^2 - s_2} = 0.00089239$$

(where  $X_1$ ,  $X_2$ ,  $s_1$ ,  $s_2$  and  $s_3$  are the same as determined for models III).

$$k_2(\text{II,IV}) = X_1 - k_3(\text{II,IV}) = 0.00417203$$

$$k_1(\text{II,IV}) = s_1 - X_1 = k_1(\text{III}) = 0.00416038$$

$$A_{12} A_{21}(\text{II,IV}) = \frac{s_1 - s_2 k_1 + k_1^2 (k_2 + k_3)}{k_1 k_2 (k_1 - k_3)} = 0.150742$$

$$A_{23} A_{32}(\text{II,IV}) = \frac{s_2 k_3 - s_3 - k_3^2 (k_1 + k_2)}{k_2 k_3 (k_1 - k_3)} = 0.632790 = 1 - \frac{X_2}{k_2 k_3}$$

Model IIa (Fig. 3):  $A_{01} = e_1 = 0$ ;  $e_2 = 1 - U = 0.01$ ;  $e_3 = U = 0.99$ ;  $s_1 = 1$ ;  $s_2 = s_3 = 0$ .

$$A_{02} = \frac{(1-U)s_3}{k_1 k_2 k_3} = 0.002165$$

$$A_{12} = A_{12} A_{21} = 0.150742$$

$$A_{32} = 1 - A_{12} - A_{02} = 0.847093$$

$$A_{23} = \frac{A_{23} A_{32}}{A_{32}} = 0.747014$$

$$A_{03} = 1 - A_{23} = 0.252986$$

$$Q_3(\text{IIa}) = \frac{k_1}{k_3} \frac{A_{32}}{(1 - A_{23} A_{32})} Q_1 = 45.9 \text{ mg (marrow iron transit pool)}$$

$$E_3(\text{IIa}) = \text{RBC Iron Turnover} = k_3 A_{03} Q_3 \times 1440 = 14.9 \text{ mg/day}$$

$$Q_2(\text{IIa}) = \frac{k_1}{k_2} \frac{1}{(1 - A_{23} A_{32})} Q_1 = 11.58 \text{ mg (extravascular labile pool)}$$

$$E_2(\text{IIa}) = k_2 A_{02} Q_2 \times 1440 = 0.1507 \text{ mg/day (iron to storage)}$$

Model IVa (Fig. 3):  $A_{02} = e_2 = 0$ ;  $e_1 = 1 - U = 0.01$ ;  $e_3 = U = 0.99$ ;  $\sigma_1 = 1$ ;  $\sigma_2 = \sigma_3 = 0$ .

$$A_{01} = \frac{1-u}{k_1 \left( \frac{a_1}{\lambda_1} + \frac{b_1}{\lambda_2} + \frac{c_1}{\lambda_3} \right)} = \frac{(1-U)s_3}{k_1 X_2} = 0.005896$$

$$A_{21} = 1 - A_{01} = 0.994104$$

$$A_{12} = \frac{A_{12} A_{21}}{A_{21}} = 0.151636$$

$$A_{32} = 1 - A_{12} = 0.848364$$

$$A_{23} = \frac{A_{23} A_{32}}{A_{32}} = 0.745894$$

$$A_{03} = 1 - A_{23} = 0.254106$$

$$Q_3(\text{IVa}) = \frac{k_1}{k_2} \frac{A_{21} A_{32}}{(1 - A_{23} A_{32})} Q_1 = 45.7 \text{ mg (marrow iron transit pool)}$$

$$E_3(\text{IVa}) = \text{RBC Iron Turnover} = k_3 A_{03} Q_3 \times 1440 = 14.9 \text{ mg/day}$$

$$Q_2(\text{IVa}) = \frac{k_1}{k_2} \frac{A_{21}}{(1 - A_{23} A_{32})} Q_1 = 11.52 \text{ mg (extravascular labile pool)}$$

$$E_1(\text{IVa}) = k_1 A_{01} Q_1 \times 1440 = 0.1507 \text{ mg/day (iron to storage)}$$

When, as in the present case, no hemolytic component is present, it is possible to calculate the expected (normalized) RBC uptake data on the basis of the approximating equation to the experimental plasma  $\text{Fe}^{59}$  disappearance data.

Since, for the duration of the experiment, the behavior of the tracer in the RBC pool is assumed to be consistent with that of a metabolite in an accumulation pool (5, 6), the theoretical RBC  $\text{Fe}^{59}$  uptake values at any time must be proportional to the corresponding integral from zero to  $\tau = t - (1/k_4)$  (where  $1/k_4$  is the estimated time lag (8)) of the tracer concentration in the marrow iron transit pool  $Q_3$ . Let  $q_3(\tau)$  denote the activity in the marrow iron transit pool and  $q_{\text{RBC}}(t = \tau + 1/k_4)$  that in the RBC pool for  $t \geq 1/k_4$ . Then, for models IIIa and IIIb

$$\begin{aligned} \frac{q_3(t, \tau)}{q_0} &= A_{31}k_1 \left[ -\frac{\lambda_1 - k_2}{(\lambda_1 - \lambda_2)(\lambda_1 - \lambda_3)} e^{-\lambda_1 \tau} - \frac{k_2 - \lambda_2}{(\lambda_1 - \lambda_2)(\lambda_2 - \lambda_3)} e^{-\lambda_2 \tau} \right. \\ &\quad \left. + \frac{k_2 - \lambda_3}{(\lambda_1 - \lambda_3)(\lambda_2 - \lambda_3)} e^{-\lambda_3 \tau} \right] \\ &= A_{31}k_1(278.156e^{-0.0001848\tau} - 207.241e^{-0.00301\tau} - 70.913e^{-0.00603\tau}). \end{aligned}$$

The total amount of activity transferred from the marrow iron transit pool to the erythron maturation pool during the interval from zero to  $\tau$  becomes:

$$\begin{aligned} q_{\text{RBC}}\left(t = \tau + \frac{1}{k_4}\right) &= A_{31}A_{03}k_1k_3 \int_0^\tau \frac{q_3(t, \tau)}{q_0} d\tau \\ &= A_{31}A_{03}k_1k_3 \left( -\frac{278.154}{0.0001848} e^{-0.0001848\tau} + \frac{207.241}{0.00301} e^{-0.00301\tau} \right. \\ &\quad \left. + \frac{70.913}{0.00603} e^{-0.00603\tau} \right) \Bigg|_0^\tau \\ &= A_{31}A_{03}k_1k_3 \times 10^5 (14.2455 - 15.0516e^{-0.0001848\tau} \\ &\quad + 0.6885e^{-0.00301\tau} + 0.117e^{-0.00603\tau}) \text{ for } \tau = t - \frac{1}{k_4} \geq 0. \end{aligned}$$

The above theoretical RBC  $\text{Fe}^{59}$  uptake,  $U_{\text{RBC}}$ , at any time  $t = \tau + (1/k_4) \geq 1/k_4$  can be normalized by expressing it as the percentage of the maximum theoretical uptake (at  $\tau \rightarrow \infty$ ), i.e., by dividing  $q_{\text{RBC}}(t)$  by  $14.2455A_{31}k_1k_3$  and by multiplying the result by 100:

$$\begin{aligned} U_{\text{RBC}}(t, \text{IIIa, IIIb}) &= 100 - 105.66e^{-0.0001848\tau} + 4.83e^{-0.00301\tau} \\ &\quad + 0.83e^{-0.00603\tau} \quad (\tau = t - (1/k_4) \geq 0, \text{ in minutes}), \end{aligned}$$

or

$$\begin{aligned} U_{\text{RBC}}(t, \text{IIIa, IIIb}) &= 100 - 105.66e^{-0.29611\tau} + 4.83e^{-4.334\tau} + 0.83e^{-8.68\tau} \\ &\quad (\tau = t - (1/k_4) \geq 0, \text{ in hours}). \end{aligned}$$

Similarly, for the case of models IIa and IVa:

$$\begin{aligned} U_{\text{RBC}}(t, \text{IIa, IVa}) &= 100 - 109.91e^{-0.26611\tau} + 13.06e^{-4.334\tau} - 3.15e^{-8.68\tau} \\ &\quad (\tau = t - (1/k_4) \geq 0, \text{ in hours}). \end{aligned}$$

A comparison of these values with experimental uptake data (by superposition of corresponding semilogarithmic plots), indicates a time lag of 1.25 days (Table II and Fig. 6). In general, a time lag characterizes a unidirectionally connected sequence of vanishingly small pools (8), which in this case is represented by an erythropoietic series or "erythron maturation pool," interposed between the marrow iron transit pool and the peripheral erythrocytes. The size of the erythron

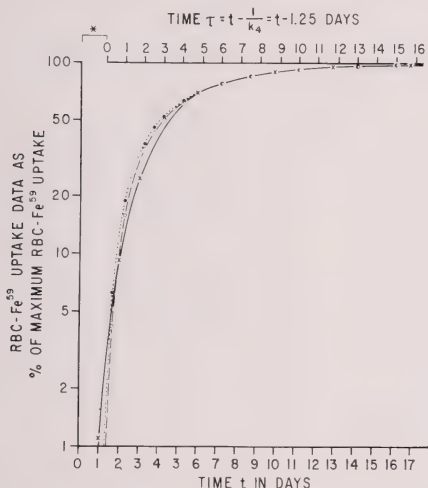


FIG. 6. Experimental RBC  $\text{Fe}^{59}$  uptake data on A.M., and their approximation by theoretical values derived from the three-pool analysis of plasma  $\text{Fe}^{59}$  disappearance data.

- ×—×—×† Experimental RBC  $\text{Fe}^{59}$  uptake (lower time scale  $t$ )
- · · · · † Theoretical RBC uptake for models IIIa and IIIb (upper time scale  $\tau = t - 1.25$  days)
- † Theoretical RBC  $\text{Fe}^{59}$  uptake for models IIa and IVa (upper time scale  $\tau = t - 1.25$  days)

\* The bracket refers to time lag  $= 1/k_4 = 1.25$  days.

† After day 5 all three lines coincide.

maturation pool is equal to RBC iron turnover multiplied by the time lag  $= 14.9 \times 1.25 = 18.6$  mg (8). Finally, consider the activity detected by the in vivo counter over an erythropoietic site. After about 12 hours the activity due to the extravascular pool becomes negligible. Therefore, when, as in the present example, no appreciable amount of  $\text{Fe}^{59}$  goes into the stores, the in vivo activity after correction for circulating blood background (2) is proportional (after the first day) to the sum of 1) the activity in the marrow iron transit pool  $q_3(t)$  and 2) the cumulative activity released from the marrow iron transit pool during the preceding time interval of one "time lag,"  $1/k_4$  (in this particular

case of 1.25 days),

$$A_{03}k_3 \int_{\max(0, t-1.4)}^t q_3 dt.$$

TABLE II

*Experimental RBC  $^{59}\text{Fe}$  Uptake Data on Patient A.M., and Their Approximation by Theoretical Values Derived from the Three-pool Analysis (Models IIIa and IIIb) of Plasma  $\text{Fe}^{59}$  Disappearance Data*

Time after $\text{Fe}^{59}$ injection (in days)	$\tau = t - 1.25 d = t - \text{time lag}$ (in days)	Experimental RBC $\text{Fe}^{59}$ uptake		Percent of Maximum Theoretical RBC $\text{Fe}^{59}$ uptake at the time $t = \tau + \text{time lag} = \tau + 1.25 \text{ days}$ .	
		As counts/min/2 ml of whole blood	As % of maximum $\text{Fe}^{59}$ uptake (4350c/m/2 cc)	Models IIIa and IIIb $U_{Tb}(t = \tau + 1.25 \text{ days}) = 100$ $- 105.7e^{-0.2661\tau}$ $+ 4.83e^{-4.333\tau}$ $+ 0.83e^{-8.68\tau}$	Models IIIa and IIIb $U_{Ta}(t = \tau + 1.25 \text{ days}) = 100$ $- 109.91e^{-0.2741\tau}$ $+ 13.06e^{-4.031\tau}$ $- 3.15e^{-8.68\tau}$
0.42		27.6	0.63		
1.00		47.9	1.10		
1.60	0.35			6.4	4.2
2.00		400.6	9.2		
2.25	1.00			18.9	15.8
3.00		1074	24.7		
3.25	2.00			38.0	35.5
3.75	2.50			46.1	43.0
4.25	3.00			52.4	50.5
5.25	4.00			63.6	62.1
6.00		3047	70.0		
7.25	6.00			78.6	77.3
8.75	7.50			85.6	85.1
10.00		3918	90.1		
11.25	10.00			92.6	92.3
12.25	11.00			94.4	94.1
13.00		4150	95.4		
14.25	13.00			96.7	96.5
16.25	15.00			98.1	98.0
17.00		4280	98.4		
17.25				98.5	98.4

Using the numerical values obtained in the calculations of RBC uptake, one gets for models IIIa and IIIb:

$$\begin{aligned}
 \frac{q_3(t)}{q_0} + A_{03}k_3 \int_{\max(0, t-1.4)}^t \frac{q_3(t)}{q_0} dt \\
 = A_{31}k_1(278.15e^{-\lambda_1 t} - 207.24e^{-\lambda_2 t} - 70.913e^{-\lambda_1 t}) \\
 + A_{31}k_1(-286.61e^{-\lambda_3 t} + 13.11e^{-\lambda_2 t} + 2.24e^{-\lambda_1 t} + 286.61e^{-\lambda_3 \cdot \max(0, t-1.4)} \\
 - 13.11e^{-\lambda_2 \cdot \max(0, t-1.4)} - 2.24e^{-\lambda_1 \cdot \max(0, t-1.4)}).
 \end{aligned}$$



For  $t$  in days, the theoretical in vivo activity can be expressed as

Theoretical in vivo activity

Constant

$$= 286.61e^{-0.26611\max(0, t-1.25)} - 8.46e^{-0.2661t} - 13.11e^{-4.334\max(0, t-1.25)} \\ - 194e^{-4.334t} - 2.24e^{-8.68\max(0, t-1.25)} - 68.67e^{-8.68t}$$

The theoretical activity calculated from the above equation and multiplied by an appropriate constant is compared in Fig. 7 and Table III with the experi-

FIG. 7. Experimental in vivo radioactivity (corrected for blood background) over the bone marrow area of A.M., with 99 per cent RBC  $\text{Fe}^{59}$  uptake, and their approximation by theoretical values derived from the three-pool analysis (Models IIIa and IIIb) of plasma  $\text{Fe}^{59}$  disappearance data.

- Experimental in vivo radioactivity over bone marrow area, with 100 per cent correction for blood background.  
 •-•-• Experimental in vivo radioactivity over bone marrow area, with 99 per cent correction for blood background.  
 △-△-△ Theoretical in vivo radioactivity over any erythropoietic area.

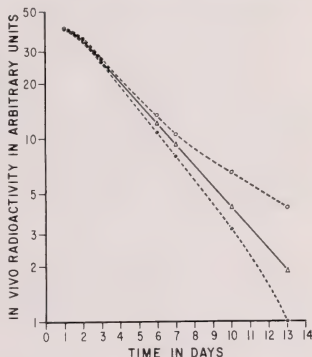


TABLE III

Experimental in vivo Counting Data (Corrected for Blood Background) over the Bone Marrow Area of Patient A.M., and Their Approximation by Theoretical Values Derived from the Three-pool Analysis (Models IIIa and IIIb) of Plasma  $\text{Fe}^{59}$  Disappearance Data

Time in days after $\text{Fe}^{59}$ injection	Experimental in vivo activity in arbitrary units over bone marrow area		Theoretical in vivo activity over bone marrow area (models IIIa and IIIb)	
	With 100% correction for circulating blood background	With 99% correction for circulating blood background	Calculated as described in the text	Calculated as described in the text and multiplied by a proportionality constant
1	39.7	39.9	262.16	40.0
2	34.8	35.2	229.30	35.0
3	26.2	27.2	176.12	26.9
6	10.8	13.4	79.26	12.1
7	8.0	10.5	60.64	9.3
10	3.2	6.5	27.55	4.2
13	1.0	4.2	12.31	1.9

mental in vivo activity over the bone marrow area, corrected for 100 per cent and 90 per cent (2) of the circulating blood background, respectively.

A comparison of the physiological constants obtained on A.M. indicates that while  $Q_2$ 's and most of  $Q_3$ 's may assume different numerical values corresponding to each of the four three-pool models considered (Fig. 3), all RBC iron turnovers,  $E_3$ 's, and three of the  $E_2$ 's and  $E_1$ 's ( $E_2$ (IIa),  $E_2$ (IIIa) and  $E_2$ (IVa)), assume equal values. These equalities are not accidental, and as is shown in the Appendix,

$$E_3(\text{IIa}) = E_3(\text{IIIa}) = E_3(\text{IIIb}) = E_3(\text{IVa}) = \frac{U s_3 Q_1}{X_2}.$$

Similarly, it can be shown that

$$E_2(\text{IIa}) = E_2(\text{IIIa}) = E_1(\text{IVa}) = \frac{(1 - U) s_3 Q_1}{X_2}.$$

Therefore, if the main purpose of the mathematical analysis of the initial three-pool system of iron metabolism is to determine the RBC iron turnover values, or the daily per cent of hemoglobin renewal, it makes no difference which of the above four alternative models is assumed. As a matter of fact, the turnover can be calculated directly from the constants of the approximating equation and the values of  $U$  and  $Q_1$ :

$$\text{RBC Fe turnover (IIa, IIIa, IIIb, IVa)} = \frac{U s_3 Q_1}{X_2} = \frac{U Q_1 \lambda_1 \lambda_2 \lambda_3}{a_1 \lambda_2 \lambda_3 + b_1 \lambda_1 \lambda_3 + c_1 \lambda_1 \lambda_2}.$$

Similarly, the total turnover of the reduced three-pool system\* for models IIa, IIIa and IVa is equal to

$$\frac{U s_3 Q_1}{X_2} + \frac{(1 - U) s_3 Q_1}{X_2} = \frac{s_3 Q_1}{X_2} = \frac{Q_1 \lambda_1 \lambda_2 \lambda_3}{a_1 \lambda_2 \lambda_3 + b_1 \lambda_1 \lambda_3 + c_1 \lambda_1 \lambda_2}.$$

From the expression  $U s_3 Q_1 / X_2$ , it follows that RBC iron turnover (at least for the above mentioned models) varies directly with  $U$  and  $Q_1$ , i.e., a given per cent error in the determination of either RBC maximum iron uptake or of the total plasma iron, introduces the same per cent error in the calculations of iron turnover. The dependence of RBC iron turnover  $= U s_3 Q_1 / X_2$  upon the approximating equation is not quite so obvious. In the preceding example (Fig. 4, Table I), the exponential function  $c_1 e^{-\lambda_2 t} = 210 e^{-0.0001848 t}$  of  $q_1' = 210 e^{-0.0001848 t} + 4200 e^{-0.000311 t} + 3030 e^{-0.0004902 t}$  ( $t$  in minutes) gives a good approximation to the experimental points corresponding to  $t = 2, t = 6$  and  $t = 10$  days, but not to  $t = 3$  days (this latter experimental point is assumed to be inaccurate). If, however, the experimental results of only three days are considered, they can be equally well approximated by

$$q_1'' = 325 e^{-0.0003565 t} + 6400 e^{-0.003706 t} + 710 e^{-0.01019 t} \quad (\text{Table I}).$$

\*That is, the total amount of iron entering and leaving the three-pool system as a whole (rather than individual pools) per unit time.

Here  $\lambda_3'' = 0.0003565$  has almost twice the value of  $\lambda_3' = 0.0001848$  of the first approximating equation, while the RBC iron turnover is increased only by about 12 per cent (Table IV). It should be noted that once the value of  $\lambda_3$  is decided upon, the determination of the corresponding  $\lambda_1$  and  $\lambda_2$  leaves little room for possible variation. Approximating the experimental plasma  $\text{Fe}^{59}$  disappearance data for the first six hours after injection by the sum of only two exponential functions, however, is not always unequivocal. In normal cases, where the disappearance rate of  $\text{Fe}^{59}$  is not excessive and the experimental curve does not exhibit a marked change in the slope on semilogarithmic paper during this initial period of six hours, equally good approximations (i.e., within the limits of experimental errors) may differ widely both with respect to the exponents and the coefficients of the two exponential functions. The respective calculated RBC iron turnovers (see Appendix A, ref. 2), however, may not differ significantly. This is illustrated in Fig. 8 and Tables V and VI for patient A.M.

The patient A.M. has a maximum RBC  $\text{Fe}^{59}$  uptake of 99 per cent and exhibits no hemolysis. Therefore, the approximation of the experimental iron 59 disappearance data for the first ten days of the experiment by a sum of three exponential functions, i.e., "the first approximation" was probably the correct one, although its deviation from a single point corresponding to  $t = 3$  days was larger than expected. The subsequent analyses of the RBC  $\text{Fe}^{59}$  uptake curve and of the in vivo activity over the bone marrow area substantiated this assumption. The "second approximation" with the exponential function  $325e^{-0.000356t}$  passing through the "off" point  $t = 3$  days, and neglecting completely the subsequent experimental results was used only as an example of relative insensitivity of RBC iron turnover values to assumed  $\lambda_3$ . When hemolysis is present, however, the experimental points after 36 or 48 hours may have to be disregarded in approximations by sums of three exponential functions. As an example, consider the plasma iron disappearance curves on two subjects with hemolytic anemia, C.L. and F.E. (Fig. 9). Here  $\circ$  and  $\bullet$  stand for the experimental plasma  $\text{Fe}^{59}$  disappearance data on subjects C.L. and F.E. respectively. The corresponding solid lines represent the theoretical approximations to these data for the first two days by the sums of three exponential functions (Table VII). Limiting such approximations to the experimental data for only the first two days is justified insofar as during this time the RBC  $\text{Fe}^{59}$  uptake (Fig. 9) is relatively small, and therefore the plasma  $\text{Fe}^{59}$  activity due to possible in vivo hemolysis of the newly formed radioactive red cells may be disregarded. The difference between the experimental and calculated plasma activities after the third day, however, becomes significant, and in the case of the subject C.L. is denoted by  $\Delta - \Delta$  (Fig. 9). The broken line approximating most of these points appears to follow closely the RBC  $\text{Fe}^{59}$  uptake values  $\square$  (though with a possible shift in time). This is the type of behavior that one would expect from the plasma radioactivity due to the random hemolysis of the red blood cells formed during the experimental period. Plasma activity due to red cell hemolysis may, several days after injection, appear as an approximately constant value. This does by no means imply the presence of a constant term in the isotopic equation of plasma  $\text{Fe}^{59}$

TABLE IV  
*Comparison of the Results of the Three-pool Analyses of the Experimental Plasma Fe<sup>59</sup> Disappearance Data (A.M.) Based on the Two Different Approximating Equations (t in Minutes)*

$$1) q' = 3030e^{-0.00663t} + 4200e^{-0.00301t} + 210e^{-0.00038t},$$

$$2) q'' = 710e^{-0.01019t} + 6400e^{-0.003706t} + 325e^{-0.000356t}.$$

	$k_1$ per minute	$k_{21}(H_a, H_b)$ $k_{21}(H_a, V_a)$ per minute	$k_{21}(H_a, H_b)$ $k_{21}(H_a, V_a)$ per minute	$Q_{21}(H_a)$ $Q_{21}(H_a)$ mg	$Q_{21}(H_a)$ $Q_{21}(H_a)$ mg	$Q_{21}(H_a)$ $Q_{21}(V_a)$ mg	$Q_{21}(H_b)$ $Q_{21}(H_b)$ mg	$\frac{E_{21}(H_a)}{E_{21}(H_b)}$ $\frac{E_{21}(H_a)}{E_{21}(V_a)}$ mg/day	$\frac{E_{21}(H_a)}{E_{21}(H_b)}$ $\frac{E_{21}(H_a)}{E_{21}(V_a)}$ mg/day	$E_{21}(H_b)$ mg/day
First Approximation $q' = 3030e^{-0.00663t}$ + $4200e^{-0.00301t}$ + $210e^{-0.00038t}$ t in minutes	0.004160	0.004778 / 0.004172	0.0002862 / 0.0008924	11.6 / 45.9	0.445 / 51.6	11.5 / 45.7	2.75 / 51.6	14.9	0.151	0.93
Second Approximation $q'' = 710e^{-0.01019t}$ + $6400e^{-0.003706t}$ + $325e^{-0.000356t}$ t in minutes	0.004790	0.009561 / 0.008432	0.0005129 / 0.001642	5.97 / 26.9	0.187 / 31.3	5.93 / 26.7	1.50 / 31.3	16.7	0.169	1.35

disappearance. More generally, in cases of instantaneous intravenous injection of  $\text{Fe}^{59}$  transferrin, the plasma  $\text{Fe}^{59}$  equations of the form  $q = \alpha_1 e^{-\lambda_1 t} + \alpha_2 e^{-\lambda_2 t} + c$  or  $q = \alpha_1 e^{-\lambda_1 t} + \alpha_2 e^{-\lambda_2 t} + \alpha_3 e^{-\lambda_3 t} + c$  (where  $c > 0$  is a constant) (9) are inadmissible. Furthermore, a subtraction of a constant value from all experimental

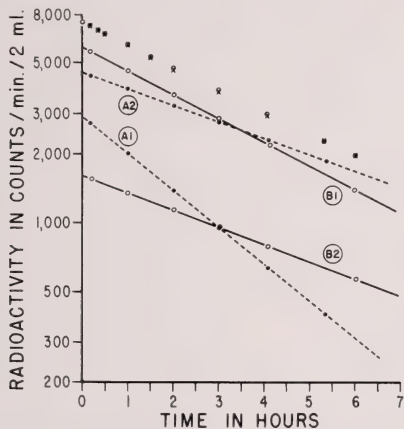


FIG. 8. Experimental plasma  $\text{Fe}^{59}$  disappearance data on A.M., during the first six hours after the tracer injection, and their approximation by sums of two exponential functions.

× × × Experimental data

--- Components of the first approximation:

$$q_1' = \alpha_1' e^{-\lambda_1' t} + \alpha_2' e^{-\lambda_2' t} = 2900 e^{-0.006188 t} + 4500 e^{-0.002772 t}$$

○ ○ ○ Second approximation:

$$q_1'' = \alpha_1'' e^{-\lambda_1'' t} + \alpha_2'' e^{-\lambda_2'' t} = 5800 e^{-0.0040 t} + 1600 e^{-0.0029 t}$$

○—○—○ Components of the second approximation:

(A<sub>1</sub>) stands for  $\alpha_1' e^{-\lambda_1' t}$

(A<sub>2</sub>) stands for  $\alpha_2' e^{-\lambda_2' t}$

(B<sub>1</sub>) stands for  $\alpha_1'' e^{-\lambda_1'' t}$

(B<sub>2</sub>) stands for  $\alpha_2'' e^{-\lambda_2'' t}$

plasma values may make it impossible to analyze the resulting curve in terms of three, rather than only two, exponential functions. It should be further noted, that in the above case the validity of the approximating equation cannot be checked directly by comparing the experimental uptake data with the theoretical data, as determined in the case of A.M., since the latter are no longer functions of the "approximating equation" alone, but depend also upon the rates of hemolysis and subsequent reutilization (after appropriate time lag) of the newly

formed circulating red cells. Such approximation of the experimental plasma  $\text{Fe}^{59}$  data, in the case of hemolytic anemia, by a sum of three exponential functions (Fig. 9, solid lines) and a component closely following the RBC  $\text{Fe}^{59}$  uptake (Fig. 9, broken line connecting  $\Delta$ - $\Delta$ ) does not take into account the return of radioactive iron from the iron stores. The experimental value for maximum RBC  $\text{Fe}^{59}$  uptake,  $U$ , on subject C.L. was 0.40 (or 40 per cent), which means that the total activity observed in the red blood cells of the patient reached

TABLE V

*Experimental Plasma  $\text{Fe}^{59}$  Disappearance Data (First Six Hours) on A.M., and Their Approximation by Sums of Two Exponential Functions ( $t$  in minutes)*

First Approximation:

$$q' = 2900e^{-0.006188t} + 4500e^{-0.002772t}$$

Second Approximation:

$$q'' = 5800e^{-0.0040t} + 1600e^{-0.0129t}$$

Column	First approximation						Second Approximation				
	1	2	3	4	5	6	7	8	9	10	11
Sampling time in min after $\text{Fe}^{59}$ injection	Experimental values: counts per min. per 2 ml of plasma	$2900e^{-0.006188t}$	$4500e^{-0.002772t}$	Theoretical values: columns 2 + 3	Deviations from experimental values: counts/min/2 ml: columns 1-4	Deviations from experimental values: per cent of theoretical values (column 4)	$5800e^{-0.0040t}$	$1600e^{-0.0129t}$	Theoretical values: columns 7 + 8	Deviations from experimental values in counts/ml/2 ml: columns 1-9	Deviations from experimental values as per cent of theoretical values (column 9)
0		2900	4500	7400			5800	1600	7400		
5	7253	2812	4438	7250	-3	0.0	5685	1577	7262	9	0.1
10	7141	2726	4377	7103	-38	-0.5	5573	1554	7127	-14	-0.2
22	6942	2531	4234	6765	-177	-2.6	5312	1501	6813	-129	-1.9
30	6573	2409	4141	6550	-23	-0.4	5144	1467	6611	38	-0.6
60	5850	2000	3811	5811	-39	-0.7	4562	1344	5906	56	0.9
90	5149	1661	3806	5167	19	0.4	4047	1232	5279	130	2.5
120	4614	1380	3227	4607	-7	-0.2	3589	1130	4719	105	2.2
180	3705	954	2732	3686	-19	-0.5	2823	949	3772	67	1.8
243	2900	645	2294	2939	39	1.3	2194	791	2985	85	2.9
318	2283	405	1864	2269	-14	-0.6	1626	636	2262	-21	-0.9
358	1988	316	1668	1984	-4	-0.2	1385	567	1952	-36	-1.8

a maximum (in this case after about 12 days) and formed a temporary plateau at 40 per cent of the total initially injected activity. This calculation of uptake does not take into account the continuous random destruction of the newly formed radioactive red blood cells and the time required for the reappearance of the activity in peripheral circulation (which varies from approximately 1.0 days, due to the "time lag," to about 12 days required to achieve "maximum uptake"). A true 80 to 100 per cent uptake would change the RBC iron turnover,  $E_{\text{RBC}}$ , and the hemoglobin renewal values for the initial three-pool model from 18.4 mg/day and 1.4 per cent per day (Tables VIII and IX subject C.L.)

to 36.8 mg/day and 2.8 per cent per day and to 46.0 mg/day and 3.5 per cent per day, respectively. Similar changes would take place for the values calculated on the basis of initial two- and one-pool systems, since in all (one- and two-pool) cases considered (Table IX) the red blood cell iron turnover and hemoglobin renewal are directly proportional to maximum  $\text{Fe}^{59}$  uptake. In addition, in the case of assumed 80 and 100 per cent uptakes, the marrow iron transit pool values  $Q_3$  (IIIa, IIIb) change from 102.0 mg to 129.6 mg and 143.4 mg, respectively, thus accounting for larger amounts of radioactivity retained temporarily in the marrow iron transit pool. Therefore, in the case of subject C.L., considerably smaller amounts of  $\text{Fe}^{59}$  than the above 40 per cent of the total injected tracer

TABLE VI

*Comparison of Some of the Results of the Two-pool Analyses of the Experimental Plasma Iron Disappearance Data (Patient A.M.) Based on the Two Different Approximating Equations (Time in Minutes)*

$$\frac{q'}{q_0} = 0.392e^{-0.006188t} + 0.608e^{-0.002772t}$$

$$\frac{q''}{q_0} = 0.784e^{-0.0040t} + 0.216e^{-0.0029t}$$

	$k_1$ (per minute)	$k_2$ (per minute)	$1 - A_{12}A_{21}$	Red cell iron turnover $k_1(1 - A_{12}A_{21})$ $\times UQ_1 \times 1440$ mg/day
First Approximation $q'/q_0 = 0.392e^{-0.006188t}$ $+ 0.608e^{-0.002772t}$ t in minutes	0.004079	0.004814	0.8730	21.65
Second Approximation $q''/q_0 = 0.784e^{-0.0040t}$ $+ 0.216e^{-0.0029t}$ t in minutes	0.00376	0.00314	0.9826	22.47

dose are transferred to the stores during the first two weeks of the experiment. If the stores are large (as is clinically probable for subject C.L.) the amount of radioactivity returned to the transport subsystem, and hence to plasma, is too small to interfere with the approximation values due to the three exponential functions of the plasma  $\text{Fe}^{59}$  disappearance data during the first two days of the experiment, though it may contribute significantly to the later component (Fig. 9,  $\Delta$ - $\Delta$ ).

The plasma  $\text{Fe}^{59}$  disappearance data on four normal subjects and seventeen patients were analyzed in terms of four different three-pool models, as illustrated above. The results of this analysis are summarized in tables VII, VIII and IX.

Table VII gives the constants of the approximating equations,

$$q_1/q_0 = a_1e^{-\lambda_1t} + b_1e^{-\lambda_2t} + c_1e^{-\lambda_3t};$$



i.e.,  $a_1$ ,  $b_1$ ,  $c_1$ ,  $\lambda_1$ ,  $\lambda_2$ , and  $\lambda_3$  as well as the relative (fractional) pool rate constants  $k_1$ ,  $k_2$  and  $k_3$  (6), for the models IIa, IIIa, IIIb, IVa (Fig. 3).

Table VIII gives the pool sizes in mg and the turnover rates  $E_3$ ,  $E_2$  and  $E_1$ , i.e., mg of iron leaving per day the three-pool system through pool  $Q_3$ ,  $Q_2$  and

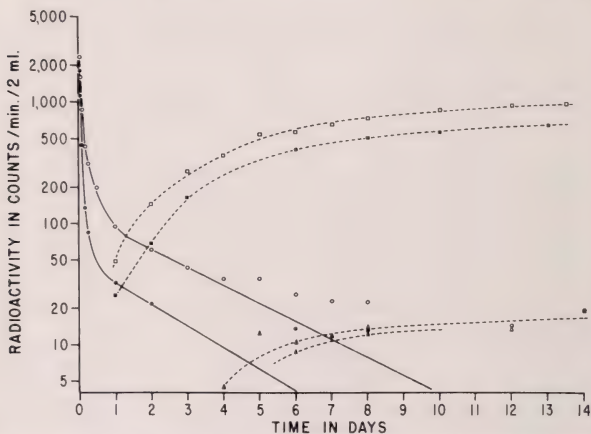


Fig. 9. Plasma disappearance and RBC  $\text{Fe}^{59}$  uptake data on two subjects with hemolytic anemia

- ○ ○ Experimental plasma  $\text{Fe}^{59}$  disappearance data on subject C.L.
  - ● ● Experimental plasma  $\text{Fe}^{59}$  disappearance data on subject F.E.
  - □ □ Experimental RBC  $\text{Fe}^{59}$  uptake data (in whole blood) on subject C.L.
  - ■ ■ Experimental RBC  $\text{Fe}^{59}$  uptake data (in whole blood) on subject F.E.
  - — — Approximations to the experimental plasma  $\text{Fe}^{59}$  disappearance data of subjects C.L. and F.E. for the first two days after the tracer injection, by means of sums of three exponential functions
- $$\alpha_1 e^{-\lambda_1 t} + \alpha_2 e^{-\lambda_2 t} + \alpha_3 e^{-\lambda_3 t}$$
- △ △ △ Experimental  $\text{Fe}^{59}$  disappearance data (after two days following the initial tracer injection) on subject C.L., minus the corresponding theoretical values of the approximating equation.
  - ▲ ▲ ▲ Experimental  $\text{Fe}^{59}$  disappearance data (after two days following the initial tracer injection) on subject F.E., minus the corresponding theoretical values of the approximating equation. Since only two points are available, the line — — — is at best an approximation.

$Q_1$ , respectively. It should be noted again that the RBC iron turnover,  $E_3$ , is always the same for all models considered.

Finally, Table IX compares the RBC iron turnover values derived on the basis of three-pool analysis with those calculated on the assumptions of two- and one-pool systems only.

It was hoped that the comparison of various pool parameters obtained on the basis of the four models considered, would unequivocally lead to the model of

$\lambda_1, \lambda_2, \lambda_3, a_1, b_1$  and  $c_1$  are constants of the corresponding approximating equation

$$\frac{q_1}{q_0} = a_1 e^{-\lambda_1 t} + b_1 e^{-\lambda_2 t} + c_1 e^{-\lambda_3 t}$$

$k_1, k_2$  and  $k_3$  are relative (fractional) rates at which iron leaves the pools  $Q_1$  (plasma pool),  $Q_2$  (extravascular labile pool) and  $Q_3$  (narrow iron transit pool) respectively. In other words, each  $k_i$  refers to the fraction of the total metabolite content of the corresponding pool leaving it per unit time (minute) for different pool models considered.

Subject	Diagnosis	Models IIIa and IIIb				Models IIa and IVa				
		$\lambda_1 \times 10^2$ per min	$\lambda_2 \times 10^2$ per min	$\lambda_3 \times 10^3$ per min	$a_1$	$b_1$	$c_1$	$k_1 \times 10^2$ per min.	$k_2 \times 10^2$ per min.	$k_3 \times 10^3$ per min.
E.A.	Normal volunteer	1.3000	0.7540	0.3100	0.6566	0.3405	0.0029	1.1104	0.9407	0.3393
J.O.	Normal volunteer	0.8600	0.5750	0.3350	0.3878	0.6094	0.0028	0.6840	0.7492	0.3525
H.H.	Normal volunteer	1.1200	0.5380	0.2460	0.3320	0.6641	0.0039	0.7292	0.9263	0.2703
A.T.	Normal volunteer	1.4200	0.6150	0.1540	0.3322	0.6645	0.0033	0.8804	1.1521	0.1784
S.H.	Cerebral accident; normal peripheral hemogram	0.8663	0.2350	0.3229	0.8824	0.0966	0.0210	0.7877	0.3003	0.4622
A.M.	Neurological disorder; normal peripheral hemogram	0.6030	0.3010	0.1848	0.4073	0.5645	0.0282	0.4160	0.4778	0.2862
E.R.	Respiratory infection; normal peripheral hemogram	3.2000	0.7970	0.5250	0.1881	0.7757	0.0362	1.2221	2.7434	0.8402
F.A.	Cirrhosis of liver; normal peripheral hemogram	1.0270	0.1936	0.1273	0.7008	0.2120	0.0272	0.7728	0.4361	0.2452
G.B.	Polycythemia vera	2.6700	0.9000	0.3187	0.5396	0.4449	0.0155	1.8417	1.7071	0.5304
C.W.	Polycythemia vera	2.6654	0.5634	0.2976	0.8830	0.1046	0.0124	2.4127	0.7932	0.5266
A.G.	Myeloid metaplasia	1.4000	0.5230	0.5635	0.7876	0.1610	0.0514	1.2605	0.6989	1.0987
L.B.	Myeloid metaplasia (spent polycythemia vera)	2.5200	0.4530	0.2406	0.3275	0.6204	0.0521	1.1077	1.8345	0.5485
L.L.	Myeloid metaplasia (spent polycythemia vera)	5.7500	0.5435	0.2620	0.8709	0.1049	0.0242	5.0653	1.1653	0.8911
M.A.T.	Myeloid metaplasia	1.5800	0.3640	0.3365	0.8703	0.1054	0.0243	1.4143	0.5033	0.6008
B.R.	Myeloid metaplasia	1.1600	0.2010	0.2238	0.9252	0.0613	0.0155	1.0836	0.2644	0.3538
R.M.	Myeloid metaplasia	3.5300	0.3400	0.1040	0.6849	0.3082	0.0039	3.1391	2.7101	0.3120
F.E.	Hemolytic anemia with hemosiderosis	1.8240	0.4418	0.2917	0.9331	0.0502	0.0167	1.7246	0.5153	0.5462
C.L.	Hemolytic anemia	1.6600	0.2400	0.2065	0.8261	0.1420	0.0319	1.4031	0.4083	0.4627
A.M.	Treated iron deficiency anemia	3.3000	0.5077	0.3440	0.8360	0.1435	0.0205	2.8324	0.9403	0.6937
W.O.	Hemochromatosis*	1.2160	0.2272	0.1781	0.5120	0.4425	0.0455	0.7239	0.7026	0.3449
S.S.	Aplastic anemia	1.7100	0.2493	0.2115	0.1070	0.7749	0.1181	0.3787	1.5509	0.5000

\* See reference 1 for comment on W.O.

TABLE VIII

Comparison of Pool Sizes and Amounts of Iron Leaving Three pool System through Various Pools per Unit Time.  
 Calculated on the Basis of Four Three pool Models (Ila, IIIa, IIIb, and IVa) for Four Normals and Seventeen Patients

$Q_p$  = plasma pool  
 $Q_{p(Ila)}, Q_{p(IIIa)}, Q_{p(IIIb)}$  and  $Q_{p(IVa)}$  refer to extravascular labile pools corresponding to the four three pool models considered.  
 $Q_{p(Ila)}, Q_{p(IIIa)}, Q_{p(IIIb)}$  and  $Q_{p(IVa)}$  refer to marrow iron transit pools corresponding to the four three pool models considered.  
 $E_{p(Ila)} = E_{p(IIIa)} = E_{p(IIIb)}$  and  $E_{p(IVa)}$  refer to the amount (mg) of "to storage" (1) iron leaving various pool systems through pools  $Q_p$  and  $Q_p$  per unit time (day).  
 $E_p = E_{p(Ila)} = E_{p(IIIa)} = E_{p(IIIb)} = E_{p(IVa)}$  = red blood cell iron turnover, or the amount (mg) of iron leaving the system through the marrow iron transit pool  $Q_p$  per unit time (day). RBC iron turnover is the same for all four pool models considered. Percent hemoglobin renewal is determined by dividing  $E_p$  by RBC volume and by multiplying the result by 100.  
 For maximum (fractional) red cell  $Fe^{59}$  uptakes ( $f^*$ ), RBC  $C_{Fe}$  volumes and red cell renewal rates see Table IX.

Subject	Diagnosis	Plasma iron pool, $Q_p$ , (mg)	Extravascular labile iron pool, ( $Q_p$ , mg)				Marrow iron transit pool, ( $Q_p$ , mg)		"Iron to storage" (mg/day)				Red cell iron turnover, $E_p$ (mg/day) Models IHa, IIHa, IIHb, and IVa
			Model IHa	Model IIHa	Model IIHb	Model IVa	Model IHa	Models IIHa and IIHb	Model IVa	$E_p$ , Model			
										$E_p$ (Model IHa)	$E_p$ (Model IIHb)	$E_p$ (Model IVa)	
F.A.	Normal volunteer	1.62	4.0	0.37	5.3	3.5	11.0	42.8	40.8	3.3	47.7	18.9	
J.O.	Normal volunteer	2.32	4.4	0.47	11.8	3.6	37.0	35.1	33.1	1.2	106	16.8	
H.H.	Normal volunteer	2.21	2.9	0.46	3.8	2.4	46.1	43.8	42.7	3.6	29.6	15.3	
A.T.	Normal volunteer	3.11	4.3	0.66	4.1	2.4	119	112	110	5.3	33.1	24.2	
S.H.	Cerebral accident; normal peripheral hemogram	2.51	12.8	2.1	9.8	10.9	24.8	29.4	22.7	4.22	20.0	13.3	
A.M.	Neurological disorder; normal peripheral hemogram	4.27	11.6	0.45	2.8	11.5	45.9	54.6	45.7	0.15	0.03	14.9	
E.R.	Respiratory infection; normal peripheral hemogram	2.47	2.1	0.30	0.82	2.4	25.1	26.3	25.1	0.00*	0.00*	20.7	
F.A.	Cirrhosis of liver; normal peripheral hemogram	3.98	10.8	3.7	6.6	9.9	58.1	60.0	54.7	3.74	6.35	9.8	
C.B.	Polycythemia vera	2.55	5.7	0.89	2.5	5.3	59.6	60.1	56.5	4.83	13.7	26.2	
C.W.	Polycythemia vera	1.22	3.3	0.86	2.5	8.1	20.9	42.8	37.2	0.00*	0.00*	18.7	
A.G.	Myeloid metaplasia	4.80	20.5	4.2	15.2	14.1	23.3	28.5	17.1	27.0	98.2	12.5	

L.B.	Myeloid metaplasia (spent polycythemia vera)	7.53	7.1	3.0	4.5	5.9	59.1	53.4	50.1	21.0	31.9	8.6
L.L.	Myeloid metaplasia (spent polycythemia vera)	1.85	12.7	4.3	5.6	12.3	42.4	48.7	41.5	3.50	4.57	17.5
M.A.T.	Myeloid metaplasia	2.42	14.2	2.3	7.0	12.6	31.5	37.7	28.9	5.72	17.3	16.6
B.R.	Myeloid metaplasia	3.01	24.3	4.4	15.2	20.7	50.5	59.2	45.6	6.93	23.9	17.2
R.M.	Myeloid metaplasia	5.10	92.2	0.98	9.8	80.3	400	428	349	29.9	299	44.7
F.E.	Hemolytic anemia with hemolysis	9.35	103	14.3	74.6	67.5	147	160	101	79.5	414	32.8
C.L.	Hemolytic anemia	8.40	39.4	15.1	24.5	33.0	95.4	102	82.8	27.6	44.5	18.4
A.M.	Treated iron deficiency anemia	0.814	40.2	1.0	1.8	3.9	17.3	19.3	17.1	0.62	1.11	9.7
W.O.	Hemochromatosis*	6.85	10.1	4.1	6.5	7.8	59.3	60.1	55.7	6.45	10.2	13.6
S.S.	Aplastic anemia	8.26	3.8	1.3	2.3	2.8	28.1	22.8	20.8	12.2	22.4	1.4

\* See reference 1.

TABLE IX

Comparison of RBC iron turnover rates (and hence of hemoglobin renewals) calculated on the basis of a) one pool system, b) two pool system, c) three pool system, on four normal subjects and seventeen patients.

The last two columns indicate the total amount and the fractional amount of iron leaving  $Q_1$  for  $Q_2$  (models IIIa, IIIb).

Subject	Diagnosis	Weight (kg)	RBC Cr <sup>59</sup> volume (ml)	U Maximum fractional red cell Fe <sup>59</sup> uptake	Red cell iron turnover (mg. day) based upon			Percent red cell renewal/day based upon			$k_{12}$ ( $Q_1$ -IIIa, IIIb) of amount of iron returned to plasma from marrow $Q_2$ which is returned to plasma
					One pool analysis	Two pool analysis	Three pool analysis	One pool analysis	Two pool analysis	Three pool analysis	
E. A.	Normal volunteer	57.7	1840	0.85	22.6	20.7	18.9	1.23	1.12	1.03	2.0
J. O.	Normal volunteer	63.6	2035	0.80	18.2	17.7	16.8	0.90	0.87	0.82	1.0
H. H.	Normal volunteer	62.3	1840	0.81	20.1	16.8	15.3	1.08	0.90	0.82	1.8
A. T.	Normal volunteer	88.2	2280	0.82	30.0	26.6	24.2	1.35	1.19	1.09	4.6
S. H.	Cerebral accident: normal peripheral hemogram	81.5	1580	0.759	20.5	17.9	13.3	1.29	1.13	0.83	6.4
A. M.	Neurological disorder: normal peripheral hemogram	70.6	1635	0.990	21.1	22.5	11.9	1.41	1.33	0.88	7.6
E. R.	Respiratory infection: normal peripheral hemogram	71.6	1900	1.000	32.9	28.8	20.7	1.73	1.51	1.09	11.7
F. A.	Cirrhosis of liver: normal pe ripheral hemogram	62.3	1200	0.725	34.3	20.9	9.8	2.86	1.74	0.82	11.1
G. B.	Polycythemia vera	82.7	3355	0.845	47.8	39.0	26.2	1.21	0.99	0.66	19.7
C. W.	Polycythemia vera	52.3	2170	1.000	39.1	27.0	18.7	1.80	1.24	0.86	13.7
A. G.	Myeloid metaplasia	47.4	1250	0.316	28.8	19.3	12.5	2.30	1.54	1.00	32.6
L. B.	Myeloid metaplasia (spent polycythemia vera)	63.6	1700	0.292	27.4	17.2	8.6	1.61	1.01	0.51	34.1
L. L.	Myeloid metaplasia (spent polycythemia vera)	71.8	2970	0.833	118.5	59.8	17.5	3.99	2.01	0.59	44.7
M. A. T.	Myeloid metaplasia	62.7	1210	0.744	36.0	25.2	16.6	2.97	2.08	1.37	16.0
B. R.	Myeloid metaplasia	77.3	1530	0.713	31.5	27.3	17.2	2.06	1.78	1.12	12.9
R. M.	Myeloid metaplasia	54.5	2155	0.599	138.5	130.2	44.7	6.43	6.04	2.08	147.6
F. E.	Hemolytic anemia with hemo siderosis	73.2	1540	0.292	64.9	48.6	32.8	4.21	3.15	2.13	93.2
C. L.	Hemolytic anemia	76.8	1320	0.400	67.2	36.5	18.4	5.09	2.76	1.39	49.6
A. M.	Treated iron deficiency anemia	61.8	993	0.940	30.6	15.2	9.7	3.08	1.53	0.98	9.6
W. O.	Hemochromatosis*	53.0	1760	0.678	38.7	22.5	13.6	2.20	1.28	0.77	16.2
S. S.	Aplastic anemia	56.4	768	0.105	3.1	2.9	1.4	0.41	0.38	0.19	15.3

\* See reference 1 for comment on W. O.

choice. Such, however, is not the case. The RBC iron turnover,  $E_3$ , is identical for all four models; the amounts of iron leaving the reduced system for the storage pools are the same for three of the four models ( $E_2(\text{IIa}) = E_2(\text{IIIa}) = E_1(\text{IVa})$ ) and are roughly proportional to the values  $E_2(\text{IIb})$  obtained on the basis of the fourth pool model IIb (neither set of values appears at present preferable to the other); finally, the pool sizes  $Q_2$  and  $Q_3$ , though different for most models, do not vary significantly from each other (with the exception of  $Q_2(\text{IIIb})$ ) and do not form at present a valid basis for separation. If, however, the main purpose is to determine the RBC iron turnover, the choice of an optimal model becomes unnecessary (see above). The RBC iron turnover,  $E_3$  (for any of the four models), can be obtained directly from  $U$ ,  $Q_1$  and the constants of the approximating equation,

$$\frac{q_1}{q_0} = a_1 e^{-\lambda_1 t} + b_1 e^{-\lambda_2 t} + c_1 e^{-\lambda_3 t}$$

$$E_3 = \frac{s_3 U Q_1}{X_2} = \frac{U Q_1 \lambda_1 \lambda_2 \lambda_3}{a_1 \lambda_2 \lambda_3 + b_1 \lambda_1 \lambda_3 + c_1 \lambda_1 \lambda_2} \text{ mg min}$$

(where  $Q_1$  is in mg., and  $\lambda$ 's are per minute).

At the beginning of this paper, several models of the initial transport sub-system were eliminated from consideration on the ground that they lead to physiologically untenable values in the two-pool analysis. That this is also true in the case of a three-pool analysis, and that therefore such elimination is justified, can be shown by applying appropriate equilibrium conditions to pool constants of groups II and IV, respectively. It follows that

$$E_3(\text{IIb}) = E_3(\text{IVb}) = \frac{1 - A_{23}A_{32}(\text{II,IV})}{A_{12}A_{21}(\text{II,IV})} E_3(\text{IIa,IVa}).$$

These results are given in Table X for nine patients.

Since no significant hemolytic components are present in these patients, the per cent hemoglobin renewal per day should fall within normal limits. This is not always the case for the models IIb and IVb, even after allowing for errors up to ten per cent each in the determinations of  $Q_1$  and  $U$ . The RBC iron turnover rates assume intermediary values for the remaining models of groups II and IV: when  $U$  is high, the RBC iron turnovers for models IIc and IVc will approximate the values of  $E_3(\text{IIa, IVa})$ , while those for models IId and IVd will approximate the values of  $E_3(\text{IIb, IVb})$ . For the sake of completeness, it can be further shown that the RBC iron turnover for the remainder of the sixteen iron transport sub-systems is the same as for the four models originally considered, namely,

$$E_3(\text{Ia}) = E_3(\text{Ib}) = E_3(\text{Ic}) = E_3(\text{Id}) = E_3(\text{IIa}) = E_3(\text{IIIa})$$

$$= E_3(\text{IIIb}) = E_3(\text{IIIc}) = E_3(\text{IIId}) = E_3(\text{IVa}) = \frac{U Q_1 s_3}{X_2}.$$

The comparison of RBC iron turnovers and per cent hemoglobin renewals de-



rived on the basis of one-, two-, and three-pool analysis, respectively, indicates that in general such results are largest for one-pool and smallest for three-pool models. This conclusion is not unexpected, insofar as the one-pool model takes no account of iron returning to plasma. Similarly, in the case of a two-pool system, though iron is assumed to interchange between plasma and an extra-vascular labile pool, no account is taken of iron returning from the marrow iron transit pool to the transport subsystem. In the case of the reduced three-pool systems corresponding to models IIIa and IIIb, such return of iron from the

TABLE X

*Comparison of RBC Iron Turnovers and Percent Red Cell Renewals Calculated on the Basis of Models IIa, IIIa, IIIb, IVa, IIb and IVb Respectively on Four Normal Subjects and Nine Patients with No Significant Hemolytic Component*

See Table IX for one- and two-pool data.

Subject	Diagnosis	RBC iron turnover in mg/day		Percent red cell renewed, day	
		$\frac{E_1(\text{IIa})}{E_2(\text{IIIb})} = \frac{E_3(\text{IVa})}{E_4(\text{IVb})}$	$\frac{E_2(\text{IIb})}{E_3(\text{IVb})}$	Models IIa, IIIa, IIIb and IVa	Models IIb and IVb
E.A.	Normal volunteer	18.9	306	1.03	16.6
J.O.	Normal volunteer	16.8	202	0.83	9.93
H.H.	Normal volunteer	15.3	81.1	0.82	4.36
A.T.	Normal volunteer	24.2	94.2	1.09	4.22
S.H.	Cerebral accident; normal peripheral hemogram	13.3	40.1	0.83	2.52
A.M.	Neurological disorder; normal peripheral hemogram	14.9	36.3	0.88	2.14
E.R.	Respiratory infection; normal peripheral hemogram	20.7	39.8	1.09	2.09
F.A.	Cirrhosis of liver; normal peripheral hemogram	9.8	14.2	0.82	1.18
G.B.	Polycythemia vera	26.2	48.5	0.66	1.23
C.W.	Polycythemia vera	18.7	35.1	0.86	1.62
A.M.	Treated iron deficiency anemia	9.7	14.1	0.98	1.43
W.O.	Hemochromatosis	13.6	18.9	0.77	1.07
S.S.	Aplastic anemia	1.4	2.0	0.19	0.27

marrow iron transit pool  $Q_3$  to plasma pool  $Q_1$  is given by  $k_3 A_{13} Q_3$  (IIIa, IIIb) in Table IX. In the last column of Table IX  $A_{13}$  (IIIa, IIIb) represents the fraction of total iron leaving  $Q_3$ , which returns to plasma pool  $Q_1$ . In analyzing the results of Table IX, it should be remembered that the RBC iron turnover (and therefore per cent red cell renewal) is directly proportional to the maximum RBC  $\text{Fe}^{59}$  uptake,  $U$ , for all cases considered, i.e., for one-, two-, and three-pool reduced systems. This means that a given error in the determination of the constant  $U$  will result in the same per cent error in the RBC iron turnover for these pool systems. In normal subjects, or subjects with no significant hemolytic component, such errors are probably well within ten per cent. This, however, is



not true for patients who exhibit considerable hemolysis (see discussion, above, on C.L.). Therefore, all RBC iron turnover and red cell renewal data on subjects A.G., L.B., B.R., R.M., F.E. and C.L. may be considerably larger than recorded in Table IX (Cf. Table XXVIII, ref. 1).

## SUMMARY

The alternative iron metabolism models discussed in the previous paper (3) are reduced to four acceptable models corresponding to the two-pool models IIa, IIIa, IIIb, and IVa (5, 6) of the iron transport subsystem. Neglecting the  $\text{Fe}^{59}$  return from active storage pool during the first few days, the  $\text{Fe}^{59}$  turnover data on patient A.M. are analyzed in detail in terms of the initial reduced three-pool systems corresponding to each of the four two-pool models considered. The results of similar calculations (including the determinations of RBC iron turnovers and various pool sizes) are tabulated for four normal subjects and seventeen patients with various blood disorders. In addition, RBC iron turnovers, calculated on the basis of initial reduced three-pool systems, are compared with those obtained from the analyses of two- and one-pool systems, respectively. Such comparisons indicated increasing RBC iron turnover values with decreasing numbers of initial pools. This is interpreted as due to the disregard of the return of radioactive iron to the two- and one-pool systems. It is further concluded that in most cases (including the four alternative reduced three-pool models IIa, IIIa, IIIb and IVa of the initial iron metabolism), the RBC iron turnover is independent of the particular model considered. It can therefore be expressed directly in terms of maximum RBC iron uptake,  $U$ , total plasma iron,  $Q_1$ , and constants of the approximating equation

$$\frac{q_1}{q_0} = a_1 e^{-\lambda_1 t} + b_1 e^{-\lambda_2 t} + c_1 e^{-\lambda_3 t};$$

$$\text{RBC iron turnover} = \frac{U Q_1 s_3}{X_2} = \frac{U Q_1 \lambda_1 \lambda_2 \lambda_3}{a_1 \lambda_2 \lambda_3 + b_1 \lambda_1 \lambda_3 + c_1 \lambda_1 \lambda_2} \text{ in mg/unit time.}$$

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## APPENDIX

To determine the RBC iron turnover in terms of  $U$ ,  $Q_1$  and the constants of the approximating equation, it is necessary to substitute the appropriate expressions (given in the calculation schemes for pool constants for different three-pool models) for the constants of  $E_3 = k_3 A_{03} Q_3$ .

In the case of Models IIIa and IIIb this becomes:

$$\begin{aligned} E_3(\text{III}) &= k_3(\text{III}) A_{03}(\text{III}) Q_3(\text{III}) = k_3 A_{03} Q_1 = k_1 Q_1 A_{03} A_{31} \\ &= \frac{k_1 U s_3 A_{31}(\text{III})}{k_1 k_2(\text{III}) k_3(\text{III}) A_{31}(\text{III})} Q_1 = \frac{U s_3 Q_1}{X_2}. \end{aligned}$$

In the case of Models IIa and IVa:

$$\begin{aligned} E_3(\text{IIa}) &= k_3(\text{II,IV}) A_{03}(\text{IIa}) Q_3(\text{IIa}) = \frac{k_3 A_{03} k_1 A_{32}}{k_3(1 - A_{23} A_{32})} Q_1 \\ &= k_1 Q_1 \frac{(1 - A_{23} A_{03})}{1 - A_{23} A_{32}} = k_1 Q_1 \frac{A_{12} - A_{23} A_{32}}{1 - A_{23} A_{32}} = k_1 Q_1 \frac{1 - A_{12} - A_{02} - A_{23} A_{32}}{1 - A_{23} A_{32}} \\ &= k_1 Q_1 \left( 1 - \frac{A_{12} A_{21} + A_{02}}{1 - A_{23} A_{32}} \right) = k_1 Q_1 \left( 1 - \frac{A_{12} A_{21} + \frac{(1 - U) s_3}{k_1 k_2 k_3}}{1 - A_{23} A_{32}} \right) \\ &= k_1 Q_1 \left( 1 - \frac{A_{12} A_{21}}{1 - A_{23} A_{32}} - \frac{(1 - U) s_3}{k_1 k_2 k_3 (1 - A_{23} A_{32})} \right). \end{aligned}$$

Since

$$k_3(\text{II,IV}) (k_1(\text{II,IV})) = \frac{X_2}{1 - A_{23} A_{32}(\text{II,IV})},$$

this becomes

$$E_3(\text{IIa}) = k_1 Q_1 \left( 1 - \frac{A_{12} A_{21}(\text{II,IV})}{1 - A_{23} A_{32}(\text{II,IV})} - \frac{(1 - U) s_3}{k_1 X_2} \right).$$

Similarly:

$$E_3(\text{IVa}) = k_3(\text{II,IV}) A_{03}(\text{IVa}) Q_3(\text{IVa}) = k_3 A_{03} \frac{k_1 A_{21} A_{32}}{k_3 (1 - A_{23} A_{32})} Q_1$$

$$\begin{aligned}
&= k_1 Q_1 \frac{A_{03} A_{21} A_{32}}{1 - A_{23} A_{32}} = k_1 Q_1 \frac{A_{21} A_{32} - A_{21} A_{23} A_{32}}{1 - A_{23} A_{32}} \\
&= k_1 Q_1 \frac{A_{21} (1 - A_{12}) - A_{21} A_{23} A_{32}}{1 - A_{23} A_{32}} = k_1 Q_1 \frac{1 - A_{01} - A_{12} A_{21} - (1 - A_{01}) A_{23} A_{32}}{1 - A_{23} A_{32}} \\
&= k_1 Q_1 \frac{1 - A_{01} - A_{12} A_{21} - A_{23} A_{32} + A_{01} A_{23} A_{32}}{1 - A_{23} A_{32}} \\
&= k_1 Q_1 \left( 1 - \frac{A_{12} A_{21}}{1 - A_{23} A_{32}} - A_{01} \right) \\
&= k_1 Q_1 \left( 1 - \frac{A_{12} A_{21} (\text{II, IV})}{1 - A_{23} A_{32} (\text{II, IV})} - \frac{(1 - U) s_3}{k_1 X_2} \right) \quad \text{as in the case of}
\end{aligned}$$

Model IIa.

Hence,

$$\begin{aligned}
E_3(\text{IIa, IVa}) &= Q_1 \left[ k_1 - \frac{k_1 A_{12} A_{21} (\text{II, IV})}{1 - A_{23} A_{32} (\text{II, IV})} - \frac{(1 - U) s_3}{X_2} \right] \\
&= Q_1 \left[ k_1 - \frac{k_1 k_2 k_3 (\text{II, IV}) A_{12} A_{21} (\text{II, IV})}{X_2} - \frac{(1 - U) s_3}{X_2} \right].
\end{aligned}$$

Substituting the appropriate expressions for  $A_{12} A_{21} (\text{II, IV})$  and  $X_2$  one gets

$$\begin{aligned}
E_3(\text{IIa, IVa}) &= Q_1 \left[ k_1 - \frac{k_1 k_2 k_3 \left( \frac{s_3 - s_2 k_1 + k_1^2 (k_2 + k_3)}{k_1 k_2 (k_1 - k_3)} \right)}{X_2} - \frac{(1 - U) s_3}{X_2} \right] \\
&= Q_1 \left[ \frac{k_1 (k_1 k_2 k_3 - k_2 k_3^2 - s_2 k_3 + s_3 + k_1 k_3^2 + k_2 k_3^2)}{X_2 (k_1 - k_3)} \right. \\
&\quad \left. - \frac{k_3 (s_3 - s_2 k_1 + k_1^2 k_2 + k_1^2 k_3)}{X_2 (k_1 - k_3)} - \frac{(1 - U) s_3}{X_2} \right] \\
&= Q_1 \left( \frac{s_3}{X_2} - \frac{(1 - U) s_3}{X_2} \right) = \frac{Q_1 U s_3}{X_2}.
\end{aligned}$$

Hence

$$E_3(\text{IIa}) = E_3(\text{IIIa}) = E_3(\text{IIIb}) = E_3(\text{IVa}).$$

Similarly it can be shown that  $E_2(\text{IIa}) = E_2(\text{IIIa}) = E_1(\text{IVa})$ .

In particular

$$\begin{aligned}
E_2(\text{IIIa}) &= k_2 (\text{III}) A_{02} (\text{III}) Q_2 (\text{III}) = k_2 A_{02} \frac{k_1}{k_2} A_{21} Q_1 \\
&= k_1 Q_1 (1 - A_{12}) A_{21} = k_1 Q_1 (A_{21} - A_{12} A_{21}) \\
&= Q_1 \frac{k_1 (1 - U) s_3}{k_1 k_2 k_3} = \frac{(1 - U) s_3 Q_1}{X_2}.
\end{aligned}$$

Hence for models IIa, IIIa and IVa, the total iron turnover

$$= Q_1 \left[ \frac{U_{s_3}}{X_2} + \frac{(1 - U_{s_3})s_3}{X_2} \right] = \frac{s_3 Q_1}{X_2}.$$

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# Studies in Iodine Metabolism

## I. Initial Miscible Iodide Pool\*

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The last fifteen years have witnessed considerable advances in the investigation of iodine metabolism. The improved isotopic tracer techniques have made it possible to follow  $I^{131}$  disappearance (as iodide) from plasma, as well as its excretion in urine and uptake by the thyroid gland (5, 6, 18, 44, 46, 55, 63, 66, 70, 71, 72, 79, 85, 108). Methods for determining " $I^{131}$  dilution space" and urinary and thyroidal  $I^{131}$  plasma clearances have been devised (6, 40, 52, 62, 65, 79). The appearance of protein bound  $I^{131}$  in plasma was measured quantitatively (5, 8, 9, 21, 35, 39, 40, 67, 79, 99) and the "dilution distribution spaces" and rates of degradation of both endogenously and exogenously labelled thyroid hormone were determined (2, 5, 21, 27, 40, 47, 79, 83, 102, 103). Most of these measurements found direct clinical application in delineating various thyroidal states. Further work combining tracer and electrophoretic techniques in the study of hormonal interactions (principally sodium-1-thyroxine and triiodothyronine) and cellular, as well as extracellular (TBG, TBPA, etc.) (25, 26, 40, 75, 80, 83) thyroxine-binding proteins, is in progress. This wealth of information, however, fails to give an overall picture of iodine dynamics. Most of the above studies and observations are applicable to only a limited phase of iodine metabolism at a time, i.e., to "iodide space" or to the rate of hormonal degradation, etc.

It is the purpose of this series of papers to develop the tools or methods which would allow us to treat the dynamic aspect of iodine metabolism as a single entity or a unified whole. Such a plan implies the exact formulation of a mathematical model or models which would reflect the dynamics of iodine metabolism (i.e., of iodine distribution and movement), as revealed in tracer experiments. Several mathematical iodine metabolism models have been formulated by G. L. Brownell (9, 10), T. H. Oddie (68, 69), and others (5, 13, 30, 85). In most of these models, however, various component pools (such as all iodide or all extra-thyroidal thyroxine compartments) are lumped together. Such models cannot give good approximations to experimental data and fall short of the desired goal.

As in the case of plasma  $Fe^{59}$  disappearance curves (74, 91, 94), plasma radioiodide and plasma radiothyroxine disappearance data can be best approximated by sums of exponential functions (89, 90, 92, 93). The general theory of formulation of corresponding mathematical pool systems, as well as the mathematics

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necessary to handle such models are discussed at length in "The multiple-pool analysis in tracer studies of metabolic kinetics" (92). For convenience, the necessary hypotheses and definitions (with special emphasis on three-pool systems of iodide and extrathyroidal thyroxine metabolism) are summarized in the fourth paper of this series (90). The resulting pool concepts are general and apply to any system subject to the postulated conditions. Before the final formulation of a multiple-pool model (or models) of a given metabolic system can take place, certain specific additional assumptions and information about the number, anatomical distribution and mutual interactions of various component pools of the metabolite must be established. In the case of iron metabolism, such data were accumulated over a period of years by various workers in the field (34, 73, 74, 91, 95, 110). The particular problems which form the subject matter of these studies in iodine metabolism are as follows:

1. Identification and "anatomical" delineation of the initial miscible iodide pool, i.e., of the compartment including plasma and red cell mass, through which the intravenously injected radioiodine tracer is "instantaneously distributed."

2. and 3. Approximation by appropriate three-pool models of the (2) inorganic and (3) protein-bound phases of iodine metabolism, respectively, as well as tentative identification of the various compartments involved.

4. Simultaneous investigation of inorganic and protein-bound phases of iodine metabolism by means of a specially developed double isotope counting technique (17).

When tracer doses of radioactive or labelled metabolites, such as sodium  $I^{131}$  as iodide,  $I^{131}$ -labelled sodium-L-thyroxine or  $Fe^{59}$ -transferrin, are injected intravenously, and followed by serial sampling of plasma radioactivity, the initial distribution space of such substances can be derived from the knowledge of the initial radioactivity of the injected material. The initial plasma radioactivity can be determined by extrapolating experimental plasma radioactivity to zero time (i.e., time of injection). In the case of  $Fe^{59}$ -transferrin and  $I^{131}$ -labelled sodium-L-thyroxine, the distribution volumes of the initial miscible accessible iron or thyroxine pools approximate that of total plasma (79, 94). This, however, is not the case when radioiodide is injected intravenously as sodium  $I^{131}$ . (To prevent the formation of elemental iodine,  $I_2$ , and hence the binding of  $I^{131}$  to plasma proteins, the injection dose is prepared in saline containing minute amounts of  $NaHSO_2$ .) When the total injected  $I^{131}$  activity is divided by the extrapolated activity per milliliter of plasma at zero time, the resulting "extended plasma" volume (i.e., the distribution volume of the initial miscible iodide pool) exceeds the combined volumes of plasma and red cell mass. The ratio of  $I^{131}$  activity per unit volume in red cells to that in plasma assumes a constant value of approximately 0.68 (61) within the first minute after injection. Such quick equilibration may, for practical purposes, be labelled as "instantaneous," indicating that both plasma and red cell mass participate in the same initial miscible iodide pool. The average plasma and red cell values per kilogram of weight in normal male and female subjects are assumed to be 43.1 34.6 and 41.5 24.6,

respectively\* (22). Using the  $I^{131}$  activity ratio of red cells to plasma,  $R = 0.68$ , we can calculate the average amount of the "extended plasma" volume which is derived from plasma and red cells: this becomes  $43.1 + 0.68 \times 34.6 = 66.6$  ml/kg and  $41.5 + 0.68 \times 24.6 = 58.2$  ml/kg for male and female subjects, respectively. If we allow for 30 per cent (107) variation in either direction, we get a range of 40.5–88.6 ml/kg. Table I gives the "extended plasma" volume in nine euthyroid subjects; these values fall well outside of the calculated normal range for plasma and red cells.

To demonstrate that the apparently large size of the initial miscible iodide pool, or the "extended plasma," is not an artifact due to the introduction into the plasma of "exogenous" inorganic sodium iodide, the following plasma  $I^{131}$  disappearance experiments were performed:

1. In three cases, radioiodide was incubated for 30 minutes with patient's plasma, prior to injection.

TABLE I

*Determination of "Extended Plasma" Volume on Nine Euthyroid Subjects*

Subject	"Extended plasma" in ml	Volume in ml/kg
A.C.	8490	125
L.C.	8640	115
C.D.	4500	95
R.M.	8040	133
V.M.	7170	109
A.M.	7530	87
A.O.	6260	100
R.P.	4740	91
P.Z.	7950	135

2. In three cases, the  $I^{131}$  injection mixture was obtained by giving large oral dosages of  $I^{131}$ , as iodide (such as for radiation thyroidectomy in angina pectoris, etc.) to donors, and collecting radioactive blood samples 30–45 minutes later. The latter were centrifuged under sterile conditions and the plasma used as " $I^{131}$  injection mixtures" for suitable recipients. For convenience, such iodide radioactivity will be referred to as "endogenous  $I^{131}$ ," in contradistinction to "exogenous  $I^{131}$ " in saline solution. The initial miscible iodide pools calculated in terms of "extended plasma" volumes on the basis of the last two experiments do not differ significantly from those determined after an intravenous injection of "exogenous  $I^{131}$ ."

In an effort to delineate the "anatomical" extent of the initial iodide pool, i.e., of the compartment throughout which the radioiodide is "almost" instantaneously distributed before it begins to penetrate into the rest of the body water,

\* In the above, the red cell values were obtained from plasma values by means of the hematocrits and may, therefore, be exaggerated.



the following simultaneous plasma and lymph experiments were performed first on a series of dogs, and later on four patients. In each case the thoracic duct was cannulated (in dogs according to the procedure described in *Experimental Surgery* by J. A. Markowitz, J. Archibald, and H. G. Downie (54), (page 683); in humans according to the method of Linder and Blomstrand (16, 50)) prior to the intravenous injection of radioiodide, and serial blood samples were withdrawn at convenient intervals of time. Since lymph was flowing continuously, lymph samples were collected at first over successive one- or two-minute intervals, followed later by intermittent two- or five-minute collections. Blood and lymph sampling covered the period from zero time to 120–150 minutes. Both lymph and either plasma or whole blood samples were then measured and counted in the standard manner in a well-type scintillation counter. In the case of lymph,

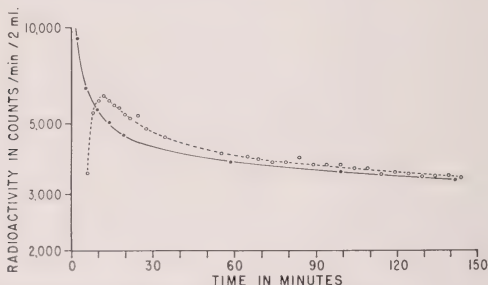


FIG. 1. Radioactivity in plasma and lymph of a dog after an intravenous injection of  $I^{131}$  as iodide, following the thoracic duct cannulation.

●—●—● Radioactivity in plasma  
○- - -○ Radioactivity in lymph

the time which had elapsed between the  $I^{131}$  injection and the middle of the collection period was corrected for the time lag due to the lymph contained in the polyethylene catheter. This was done by measuring the flow rate of the lymph and the inner volume of the tubing. The plasma  $I^{131}$  disappearance data and the corresponding (corrected for time)  $I^{131}$  appearance and disappearance values in central lymph are plotted against time for one dog and for one representative human subject in Figures 1 and 2, respectively. In dogs, the maximum activity in lymph is usually achieved after 8 to 12 minutes, soon after which both plasma and lymph radioactivity curves become parallel on semilog paper, with lymph activity approximating that of plasma. In man, the time of maximum radioactivity in lymph is achieved somewhat later, and is approximately 15 to 25 minutes in the case of the human subject in Figure 2. These results exclude central lymph as a possible participant in the initial miscible iodide pool. Whether the iodide in the peripheral lymph beds exhibits the same dynamic behavior as that in plasma and red cells, or whether it forms a part of the same compartment

as central lymph, remains to be investigated and is a problem beyond the scope of this study.

It has long been known that tissues other than the thyroid gland have the

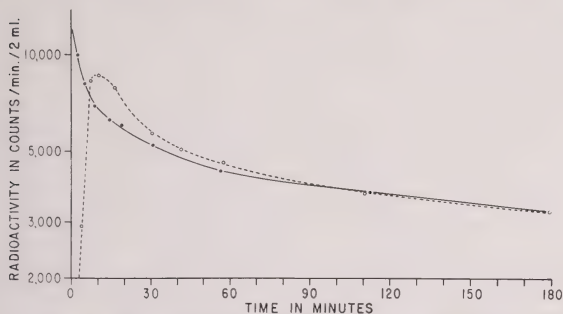


FIG. 2. Radioactivity in plasma and lymph of subject R.P., after an intravenous injection of  $I^{131}$  as iodide, following the thoracic duct cannulation.

•—•—• Radioactivity in plasma.  
○—○—○ Radioactivity in lymph.

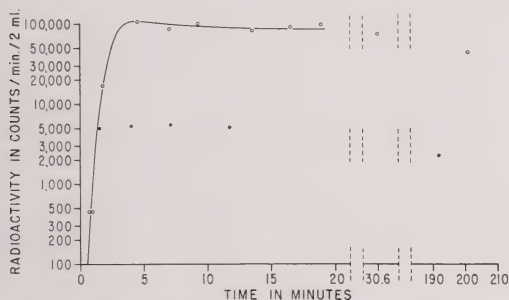


FIG. 3. Radioactivity in plasma and in gastric juice of the normal subject R.V., following an intravenous injection of  $I^{131}$  as iodide.

•—•—• Radioactivity in plasma.  
○—○—○ Radioactivity in gastric juice.

The difference in the initial Plasma  $I^{131}$  disappearance data in Figures 2 and 3, is due to a perivascular leakage of tracer in subject R.V. during the injection period.

ability to concentrate iodide (11, 29, 32, 33, 51). Riggs in "Iodine Metabolism in Man" (79) assumes that all the iodine in the body is contained in three separate compartments, the "inorganic iodide" compartment containing "all the inorganic iodide in the body, including the iodide in the secretions of the alimen-

tary tract, the iodide in red blood cells, and iodide which is present in the thyroid gland, and which has not yet been incorporated into protein." It is generally assumed that the appearance of radioiodide in gastric mucosa secretion, saliva and thyroid gland, though rapid, is not "instantaneous." For the purpose of multiple-pool analysis, however, such appearance of the radioactivity in any part of the initial iodide pool after  $I^{131}$  injection is "instantaneous" (88, 92) if its maximum activity is attained during the mixing time of  $I^{131}$  in plasma, and if its subsequent steady-state behavior parallels closely the activity in plasma. According to Honour, Myant and Rowlands (32) the maximum ratio of activity in gastric juice to that in plasma does not usually occur until at least 30-60 minutes after the intravenous  $I^{131}$  injection. Our own observations indicate that the maximum  $I^{131}$  activity (not ratio) in gastric juice is achieved within three to eight minutes (Fig. 3). This initial delay could be attributable to purely mechanical factors incident to the collection of the gastric samples. The increase in the ratios of activity in gastric juice to activity in plasma with time is fully discussed by Honour, Myant and Rowlands (32) in terms of changes in the "dead space not drained directly by the stomach tube." Once the gastric juice has been secreted from the mucosal surface, it can no longer contribute to the initial miscible iodide pool—in the sense that its egress is not readily reversible.

The same considerations apply to iodide initially trapped by other iodine concentrating tissues (thyroid, salivary glands (11, 53, 79), kidney (52, 55), and possibly bronchial mucosa) after its release from the trapping site into an area outside the plasma space (analogous to the lumen of the stomach, the thyroid follicle, the ducts of the salivary glands, the urinary collecting system, possibly the tracheobronchial tree, choroid plexus, etc.). From *in vivo* (26) and *in vitro* (14, 15, 79) studies of the kinetics of inhibition of iodide trapping, one may postulate that such iodide concentrating sites form a part of the initial miscible iodide pool in the sense that there is an "instantaneous" equilibrium with the plasma, perhaps through the mediation of some rapidly equilibrating interstitial fluid.\*

The quantitative contribution of each of the iodide-trapping sites, in spite of a high local concentration, may be small compared with iodide in plasma and red cells, but the cumulative effect of all such tissues, although exact numerical values are not available, may be considerable.

In summary, the initial miscible iodide pool consists of plasma iodide, red cell iodide, iodide in the trapping sites, and possibly an anatomically undefined volume of rapidly equilibrating interstitial fluid.

#### ACKNOWLEDGMENT

The authors wish to acknowledge the help of Dr. Charles Witte and Dr. David Kavee who rendered valuable assistance in the surgical experiments on animals.

\* By rapidly, we mean equilibration where time lag cannot be detected by presently available techniques.

## Studies in Iodine Metabolism

### II. Three-Pool Systems of Iodide Kinetics\*

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In trying to formulate a multiple-pool model of normal iodide metabolism, it is important to realize that one is not dealing with a steady-state system. While the iodide derived from (90) the hormonal degradation may be released into the iodide subsystem† at a constant absolute rate, the iodide ingested with the food is introduced in various amounts at discrete intervals of time. As a rule, the average daily intake of iodide exceeds the amount of endogenously derived metabolite. As an example, consider the case where 50  $\mu\text{g}$  of iodide are released daily from the degraded hormone and 7.5  $\mu\text{g}$  are excreted as thyroxine in feces (40), and that only 35 per cent of iodide leaving the inorganic system is taken up by the thyroid gland, while the remaining 65 per cent is excreted in urine. The average daily iodide ingestion can then be calculated as follows: total amount of iodide utilized for hormone formation is equal to the amount of iodine in the thyroxine which is degraded and excreted = 50  $\mu\text{g}$  + 7.5  $\mu\text{g}$  = 57.5  $\mu\text{g}$  = 35 per cent of iodide leaving the inorganic iodide system per day. Hence, the daily amount ingested is equal to the amount leaving the iodide system minus the iodide derived from hormonal degradation, i.e.,  $57.5 \times (100/35) - 50 = 114.3 \mu\text{g}$ .

The tracer behavior of such a non steady-state system is amenable to a mathematical analysis in terms of linear sums of exponential functions, only if it is a first order system. This means that all relevant reactions or transfer rates are first order processes and must be proportional to the amount of the metabolite in the compartment or pool from which such reactions or transfers originate. That the inorganic iodide metabolism is subject to this law follows in part from the fact that plasma  $\text{I}^{131}$  disappearance data can be approximated (during the first 10 hours of the experiment) by a sum of three exponential functions

$$\alpha_1 e^{-\lambda_1 t} + \alpha_2 e^{-\lambda_2 t} + \alpha_3 e^{-\lambda_3 t}.$$

regardless of the initial fasting state of the subject or the subsequent times at which meals are served to the patients (though no blood specimens should be drawn immediately following water or food ingestion). On the other hand, there exists independent experimental evidence supporting the contention that iodide metabolism is a first order reaction system. Riggs (79) describes an experiment

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† The system describing iodine metabolism can be separated into three distinct parts: Iodide subsystem, thyroxine in the thyroid gland and extra-thyroidal thyroxine subsystem

where renal excretion of iodide is measured repeatedly over a period of more than 30 hours after a meal. After 15–20 hours, the rate of excretion approaches a fairly constant value. If this asymptotic secretion value is subtracted from preceding experimental values, the resulting data form a straight line (corresponding to a single exponential function) on the semilog paper. This would indicate a renal excretion rate proportional (within physiological limits) to the sum of the iodide remaining in the system from the initial ingestion and the "steady-state" iodide (the constant amount of iodide which is present as the result of the degradation of thyroxine).

According to D. S. Childs et al (12), an addition of  $I^{127}$  carrier (up to 2 gm) has no effect upon "the proportional renal excretion rate of iodide," and in most of their thyroid uptake studies, the dose of  $I^{127}$  in the range of 0 to 0.1 mg "made little difference in the results." The lack of a steady-state, or dynamic equilibrium, in the metabolism of iodide makes it impossible to determine the size of any of the component pools at a given time from the knowledge of the  $I^{131}$  distribution in various iodide pools, the exact amounts of iodide lost to the thyroid gland and to the kidneys, and the iodide acquired through the degradation of the hormone. Even the relative sizes of the component pools, though less variable (except, possibly, immediately after ingestion of iodide), are subject to constant changes. Therefore, the best that can be hoped for is an "average" value of the initial miscible iodide pool in terms of  $\mu\text{g}$ , which would be the value of the pool under the conditions of a steady-state, i.e., if the total ingested iodide entered the system at a constant rate. The amounts of iodide in other component pools would follow of necessity from assumed equilibrium conditions. It should be further noted that iodide concentration in plasma may not, in general, be proportional to the total amount of iodide in the initial miscible iodide pool (except possibly during a period of fasting), and that the size of such an "extended plasma" pool (87) may vary with time after each ingestion of iodide. This may reflect the fact that the  $I^{131}$  concentration ratios of gastric juice and possibly gastric mucosa to plasma (29, 31, 32) and of saliva to plasma (11, 32) decrease considerably (as much as tenfold and more) after large dosages of  $I^{127}$  carrier. Under such conditions, the contribution to the "extended plasma" volume of iodide by the gastric mucosa, salivary glands and other presumed iodide concentrating tissues may differ significantly. This can be restated by noting that the magnitude of the initial miscible iodide pool expressed in  $\mu\text{g}$  may vary inversely with the volume of the "extended plasma" pool after a massive dose of  $I^{127}$  carrier. Subsequently, we refer to the magnitudes of the component pools of iodide metabolism in terms of their "average" contents of iodide, i.e., in micrograms.

In studying the dynamics of iodide metabolism, the obvious initial approach is to inject intravenously a tracer dose,  $q_0$ , of radioiodide and to follow the  $I^{131}$  disappearance from plasma by serially sampling the subject's blood or plasma (for radioactivity). Additional information can be obtained by simultaneous *in vivo* counting over various body sites and by determining the accumulation of radioactivity in urine.

The experimental procedures used in this study are standard. The injection dose, in case of "exogenous iodide" (87), was prepared by diluting sodium iodide ( $I^{131}$ ) with saline to an approximate activity of  $15 \mu\text{c}$  ml. After sterilization, and just prior to injection, small amounts of  $\text{NaHSO}_3$  were added to the injection mixture to prevent formation of elemental iodine ( $5-10 \text{ mg}$  are more than sufficient for  $10 \text{ cc}$  of the dose). A  $3 \text{ cc}$  dose (containing  $40-50 \mu\text{c}$  of  $I^{131}$ ) was injected intravenously. The bulk of the activity was administered during the period of  $5-10$  seconds. Quantitative delivery of the dose was ensured by appropriate techniques. Blood was withdrawn before injection, and (from the opposite arm) at precisely timed intervals of approximately  $2, 4, 6, 10, 20, 40, 80, 120, 180, 240, 300, 360, 420, 510,$  and  $600$  minutes after the  $I^{131}$  injection, the whole experiment lasting about ten hours. Two ml. samples of plasma or whole blood were counted in the usual way in a well-type scintillation counter. Whole blood counting was done only on euthyroid patients where no appreciable amount of protein bound thyroxine appeared in the circulation during the first ten hours. The resultant activities could be recalculated for plasma either by establishing a plasma-whole blood  $I^{131}$  ratio, or by determining the patient's hematocrit and using the corrections suggested by Myant et al (61) which closely correspond to our own observations. Standards were prepared by diluting the injection dose  $1:2000$ ; again  $2 \text{ ml}$  samples were used. Urine specimens were timed, diluted if necessary, and  $2 \text{ ml}$  samples counted as before. To reduce the counting error to a reasonable minimum (below  $1\%$ ),  $25,600$  counts were taken on all samples (counts per minute per  $2 \text{ ml}$  sample ranged from  $500$  to  $12,000$  with a background of about  $220$ ).

In several cases, *in vivo* counts were taken simultaneously over various body areas, usually over the period of two to three hours.

The patients were either fasting or had their breakfast at least two to three hours prior to the  $I^{131}$  injection. After the first two hours of the experiment, they were allowed to eat and drink normally (except for restriction of the salt intake), though no blood samples were withdrawn immediately after any food or liquid intake.

As was mentioned in the preceding paper (87), the plasma  $I^{131}$  disappearance data can be closely approximated by a sum of three exponential functions

$$q_1 = \alpha_1 e^{-\lambda_1 t} + \alpha_2 e^{-\lambda_2 t} + \alpha_3 e^{-\lambda_3 t} *$$

This implies the interchange of iodide between at least three different iodide compartments or pools (92).

It will be assumed at the outset that the three compartments consist of the iodide in

\* The definitions of the terms used and a summary of the fundamental assumptions underlying the formulation of  $n$ -pool models of first order metabolic systems, as well as the actual formulations of solutions of such models are discussed at length in "Multiple-pool analysis in tracer studies of metabolic kinetics" (92) and in "Studies of iodine metabolism: IV" (90). The latter paper deals specifically with three pool models which are applicable directly to iodide and thyroxine subsystems of iodine metabolism and to the initial three pool subsystem of iron metabolism (94).



- 1)  $Q_1$  = iodide in the initial miscible pool,
- 2)  $Q_2$  = iodide in the central lymph, and possibly peripheral lymph beds,
- 3)  $Q_3$  = iodide in the remaining body water.

It will be further assumed that all iodide leaves the metabolic iodide system directly through  $Q_1$ , and that the dietary iodide enters the system through  $Q_1$  while that derived from the thyroxine degradation enters it through  $Q_3$  (see Figs. 1, 2, 3, and 4). These conditions will be referred to as modes of exit and entry (92), respectively. When the ratio of the fraction of the tracer dose excreted with urine to that taken up by the thyroid gland is known, and the amounts of degraded and excreted thyroxine are available, the exact "average steady state" amounts of iodide,  $E_1$ , leaving the system through  $Q_1$  and entering it through  $Q_1$  and  $Q_3$  ( $S_1$  and  $S_3$  respectively), can be calculated.\* All the above assumptions and data, however, together with the constants of the approximating equation ( $\alpha_1$ ,  $\alpha_2$ ,  $\alpha_3$ ,  $\lambda_1$ ,  $\lambda_2$  and  $\lambda_3$ ) do not suffice to determine a three-pool system in dynamic equilibrium. Two additional conditions (92) must be imposed upon the system before it can be solved completely. Since, at this point, we know very little about the absolute interchange rates between the various iodide pools, the simplest way of imposing such limiting conditions is to set two of the rates equal to zero. Three of these models are represented in Figures 1, 2 and 3, and are designated as Iodide Models I, II and III, respectively. The arbitrary choice of restricting conditions may be justified at present on the grounds of expediency. These models are by no means final, and form only the first step in the direction of a comprehensive investigation of the dynamics of iodine metabolism. The assumptions of the modes of exit and entry of iodide, as well as the two arbitrary restrictions on the mutual interchange rates, can be altered whenever deemed necessary, and new models formulated in a similar manner.

A comprehensive discussion leading to the setting up of appropriate differential equations for each model considered and their detailed solution by means of Laplace Transforms (7, 42, 109) are given in "Multiple-pool analysis in tracer studies of metabolic kinetics" (92). A summary of the assumptions and definitions, and a formulation of the general three-pool model which can be used for the derivation of the exact solution of any particular three-pool system with sufficient number of imposed constraints or conditions (92), can be found in the fourth paper of this series. We will proceed by presenting both the symbolic solutions of each model considered, and the corresponding numerical values for subject B.B., on whom the plasma radioiodide disappearance data were obtained in the manner described above. The plasma  $I^{131}$  disappearance data for B.B., were approximated by the normalized curve

$$\frac{q_1}{q} = a_1 e^{-\lambda_1 t} + b_1 e^{-\lambda_2 t} + c_1 e^{-\lambda_3 t} \\ = 0.4845e^{-0.089t} + 0.2494e^{-0.00676t} + 0.2661e^{-0.00071t}$$

as described in "Studies in Iron Kinetics IV" (94). This equation defines the

\* For a more detailed definition of the terminology used, see (92) and (90).



approximation constants as follows:

$$\begin{aligned} a_1 &= 0.4845 & \lambda_1 &= 0.089 \\ b_1 &= 0.2494 & \lambda_2 &= 0.00767 \\ c_1 &= 0.2661 & \lambda_3 &= 0.00071 \end{aligned}$$

It should be stressed that in solving the following models, the tracer  $q_0$  is always assumed to be introduced instantaneously into the pool  $Q_1$  at the time  $t = 0$ .

*Iodide Model I* (Fig. 1).

This model consists of a central accessible pool  $Q_1$ , interchanging with two mutually non-interchanging pools  $Q_2$  and  $Q_3$ .

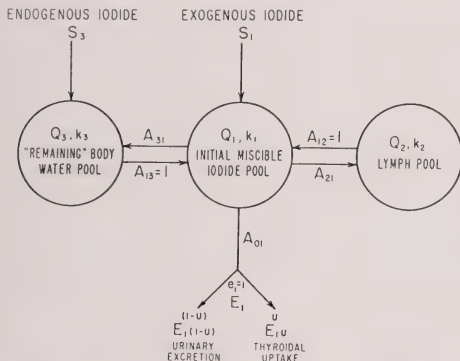


FIG. 1. Schematic representation of iodide metabolism Model I.

$Q_1$  represents the "initial miscible iodide" pool or "extended plasma" pool,  $Q_2$  and  $Q_3$  represent lymph and the remaining body water, respectively. It is assumed that all iodide,  $E_1$ , leaving the system leaves it through  $Q_1$ , and that only the ingested (or exogenous) iodide enters the system through the extended plasma pool, while the iodide derived from the degraded hormone (endogenous iodide) is released into  $Q_3$ . The characteristic polynomial of the system is given by (42, 90, 92)

$$\begin{aligned} D(p) &= \begin{vmatrix} p + k_1 & -A_{12}k_2 & -A_{13}k_3 \\ -A_{21}k_1 & p + k_2 & 0 \\ -A_{31}k_1 & 0 & p + k_3 \end{vmatrix} = (p + \lambda_1)(p + \lambda_2)(p + \lambda_3) \\ &= p^3 + (k_1 + k_2 + k_3)p^2 \\ &\quad + (k_1k_2 + k_2k_3 + k_3k_1 - A_{12}A_{21}k_1k_2 - A_{13}A_{31}k_1k_3)p \\ &\quad + k_1k_2k_3(1 - A_{12}A_{21} - A_{13}A_{31}) \\ &= p^3 + s_1p^2 + s_2p + s_3 = 0. \end{aligned}$$

When the tracer  $q_0$  is introduced instantaneously into  $Q_1$ ,

$$N_2(p) = \begin{vmatrix} p + k_1 & q_0 & -A_{13}k_3 \\ -A_{21}k_1 & 0 & 0 \\ -A_{31}k_1 & 0 & p + k_3 \end{vmatrix} = q_0 A_{21}k_1 (p + k_3),$$

and the tracer distribution equation for  $Q_2$ , or lymph, becomes

$$\frac{q_2}{q_0} = A_{12}k_1 \left[ -\frac{(\lambda_1 - k_3)e^{-\lambda_1 t}}{(\lambda_1 - \lambda_2)(\lambda_1 - \lambda_3)} + \frac{(\lambda_2 - k_3)e^{-\lambda_2 t}}{(\lambda_1 - \lambda_2)(\lambda_2 - \lambda_3)} + \frac{(k_3 - \lambda_3)e^{-\lambda_3 t}}{(\lambda_1 - \lambda_3)(\lambda_2 - \lambda_3)} \right].$$

The symmetric functions (90, 92) are:

$$s_1 = \lambda_1 + \lambda_2 + \lambda_3 = k_1 + k_2 + k_3 = 0.09647$$

$$s_2 = \lambda_1\lambda_2 + \lambda_2\lambda_3 + \lambda_3\lambda_1 = k_1k_2 + k_2k_3 + k_3k_1 - A_{12}A_{21}k_1k_2 - A_{13}A_{31}k_1k_3 = 0.6696 \times 10^{-6}.$$

$$s_3 = \lambda_1\lambda_2\lambda_3 = k_1k_2k_3(1 - A_{12}A_{21} - A_{13}A_{31}) = 0.4272 \times 10^{-6}.$$

From the definitions of the constants  $s_1, s_2, s_3, a_1, b_1, c_1, \lambda_1, \lambda_2$  and  $\lambda_3$ , the pool constants  $k_2 + k_3$  and  $k_2k_3$  can be determined as described in "Studies on iodine metabolism IV" (90) and "Multiple-pool analysis in tracer studies of metabolic kinetics" (92):

$$k_2 + k_3 = a_1(\lambda_2 + \lambda_3) + b_1(\lambda_1 + \lambda_3) + c_1(\lambda_1 + \lambda_2) = 0.051475$$

$$k_2k_3 = a_1\lambda_2\lambda_3 + b_1\lambda_1\lambda_3 + c_1\lambda_1\lambda_2 = 0.00017818.$$

Hence:

$$k_1 = 0.045995$$

$$k_2 = 0.047743$$

$$k_3 = 0.003732$$

Note that here  $k_2$  was assumed, seemingly arbitrarily, larger than  $k_3$ . The reason for this is that the maximum activity and equilibration with plasma radioactivity is achieved much sooner in  $Q_2$  than in  $Q_3$ , as will be demonstrated by direct observation of lymph activity and in vivo counting over various body sites. However, in the case of model I there are no a priori reasons why the values of  $k_2$  and  $k_3$  should not be reversed, and corresponding solutions of  $Q_2$  and  $Q_3$  with  $k_3 > k_2$  will be given below. This is not possible in the case of models II and III where  $k_3 > k_2$  does not satisfy the conditions of  $s_2$  (II, III).\*

Since in model I  $A_{12} = A_{13} = 1$  and  $e_1 = 1; e_2 = e_3 = 0$ ; it follows that

\* In the following  $s_2(X), A_{ij}(X), Q_2(X)$ , etc. will refer to  $s_2, A_{ij}, Q_2$ , etc. of the pool model X.

$$A_{21} = A_{12}A_{21} = \frac{s_3 - k_2s_3k_2^2(k_1 + k_3)}{k_1k_2(k_2 - k_3)} = 0.84116$$

$$A_{31} = A_{13}A_{31} = \frac{s_2k_3 - s_3 - k_3^2(k_1 + k_2)}{k_1k_3(k_2 - k_3)} = 0.10555$$

and

$$A_{01} = 1 - A_{21} - A_{31} = 0.05328$$

or

$$A_{01} = \frac{1}{k_1 \left[ \frac{a_1}{\lambda_1} + \frac{b_1}{\lambda_2} + \frac{c_1}{\lambda_3} \right]} = \frac{s_3}{k_1k_2k_3}.$$

Note that the last expression holds for all four models considered.\* The above solutions define only the pool constants  $k_i$  and  $A_{ii}$  (i.e., only the tracer distribution constants (92: I, Table II)), which are independent of the actual amounts of the metabolite present in, entering or leaving any of the component pools. A complete solution of this model (as well as those of the subsequent three models) can be obtained if we know the amounts of iodide entering  $Q_1$  and  $Q_3$ , respectively (i.e.,  $S_1$  and  $S_3$ ), and the fraction,  $1 - u$ , of the initial tracer dose,  $q_0$ , which leaves  $Q_1$  and is eventually excreted in urine. The fraction of  $q_0$  which eventually enters the gland can then be denoted by  $u$ . The first two data are not available for the analysis as long as we do not investigate the iodide and the thyroxine subsystems simultaneously. The best we can do under present circumstances is to assume some values available from the literature on thyroxine metabolism. We will assume, arbitrarily, the average values given by Ingbar and Freinkel (40), namely 50  $\mu\text{g}$  of iodide from daily hormone degradation and 7.5  $\mu\text{g}$  for corresponding fecal excretion, though we are aware that these values cannot be the same in all cases considered, and may be misleading. An assumption of 100  $\mu\text{g}$  and 15  $\mu\text{g}$  respectively would simply double all pool sizes and absolute transfer rates. The fraction,  $1 - u$ , of the tracer dose,  $q_0$ , excreted in urine can be determined from the comparison of cumulative radioactivity in urine at any time  $t$ , with the product  $q_0k_1A_{01}$  and the integral from zero to  $\tau$  ( $\tau = t - \text{time lag due to urine retention}$ ) of the tracer distribution function  $(q_1/q_0)(\tau)$  (i.e., with  $q_0k_1A_{01} \int_0^\tau \frac{q_1}{q_0} d\tau$ ), and from the total radioactivity of the initial dose,  $q_0$ , as illustrated in Table I. In most cases there is a time delay of about five minutes inherent in the collection of urine specimens. In the case of subject B.B., who had carcinoma of the prostate, this delay was determined to be 30 minutes by superposition of logarithmic plots of theoretical and experimental cumulative activity in urine. Since in this case, the fraction  $1 - u$  of the initially injected dose  $q_0$ , which is excreted in urine, is calculated to be 0.79, and that taken up by thyroid, or  $u$ , is equal to  $1 - 0.79 = 0.21$ , the values of  $E_1$ ,  $S_1$  and  $S_3$  can be assumed as follows:

\*  $k_1$  is the same for all these models; see below.

$$E_1 = \frac{57.5}{0.21} = 274 \mu\text{g/day}$$

$$S_3 = 50 \mu\text{g/day}$$

$$S_1 = 274 - 50 = 224 \mu\text{g/day}.$$

TABLE I

*Calculation of the Fraction,  $1 - u$ , of the Initial Injection Dose Excreted in Urine of Subject B.B.*

The total cumulative activity  $q_0 e_1(t)$  leaving the initial miscible iodide pool  $Q_1$  up to the time  $t$  is given by the integral

$$q_0 k_1 A_{01} \int_0^t \frac{q_1}{q_0} dt.$$

In the case of the subject B.B., the total radioactivity injected into the initial miscible iodide pool and eventually leaving the system is  $q_0 = q_0 e_1(\infty) = 647,000$  c/sec. A constant fraction of the above goes to the gland, while the remaining fraction is excreted in urine. At any given time  $t = \tau + 30$  (where 30 minutes is the time lag due to urine retention in the case of subject B.B.), such that  $t \geq 30$  minutes, the theoretical cumulative urine radioactivity is given by

$$\begin{aligned} (1 - u)q_0 e_1(\tau) &= (1 - u)q_0 k_1 A_{01} \int_0^\tau \frac{q_1}{q_0} d\tau \\ &= (1 - u)[647,000 - 8,400e^{-0.0089\tau} - 57,100e^{-0.00678\tau} - 581,400e^{-0.00071\tau}] \end{aligned}$$

It follows from the last column that  $1 - u = \frac{511,000}{647,000} = 0.79$  = fraction of the  $q_0$  eventually excreted in urine.

Time of urine collection (in minutes) $t$	Cumulative urine radioactivity (counts/sec)	Time corrected for the lag due to urine retention (in minutes) $\tau = t - 30$	Total cumulative theoretical radioactivity leaving iodide subsystem up to time $\tau$ (counts/sec) $q_0 e_1(\tau)$	Total cumulative theoretical radioactivity leaving iodide subsystem with urine up to time $\tau$ (counts/sec) $(1 - u)q_0 e_1(\tau) = 0.79q_0 e_1(\tau)$
75	32,300	45	41,600	32,900
130	50,500	100	76,300	60,300
192	88,100	162	109,600	86,600
242	117,200	212	133,200	105,200
322	131,800	292	166,400	131,500
$\infty$		$\infty$	647,000	511,000

These modes of exit and entry remain the same for all three models considered. Since, in the preceding, all time constants of the approximating equation are in minutes, it is expedient to express  $E_1$ ,  $S_3$  and  $S_1$  in  $\mu\text{g/min}$ . Hence we will assume

$$E_1 = 0.1904 \mu\text{g/min}$$

$$S_1 = 0.0347 \mu\text{g/min}$$

$$S_3 = 0.1556 \mu\text{g/min}.$$

The average pool sizes can then be obtained as follows:

$$Q_1 = \frac{E_1}{k_1 A_{01}} = 79.4 \mu\text{g}$$

$$Q_2 = \frac{k_1}{k_2} A_{21} Q_1 = 62.9 \mu\text{g}$$

$$Q_3 = \frac{A_{31} E_1}{k_3 A_{01}} + \frac{S_3}{k_3} = 110.3 \mu\text{g}.$$

In case of  $k_3 > k_2$ ,  $A_{01}$  and  $Q_1$  remain the same, the values of  $A_{21}$  and  $A_{31}$  become reversed and the other two pool sizes become  $Q_2 = 101.0 \mu\text{g}$  and  $Q_3 = 63.6 \mu\text{g}$ , respectively.

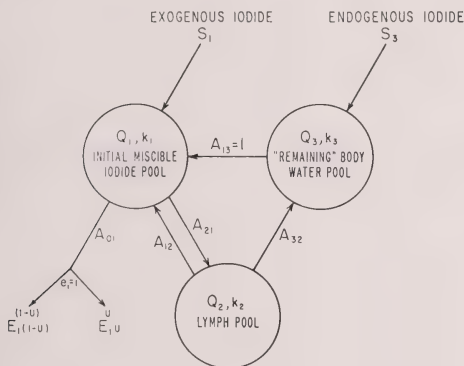


FIG. 2. Schematic representation of iodide metabolism Model II.

### *Iodide Model II (Fig. 2).*

Here the accessible pool  $Q_1$  mutually interchanges only with the lymph pool  $Q_2$ , while the transfer from  $Q_2$  to  $Q_3$  and from  $Q_3$  to  $Q_1$  is assumed unidirectional, i.e.,  $A_{23} = A_{31} = 0$  and  $A_{13} = 1$ . The corresponding characteristic polynomial becomes

$$\begin{aligned} D(p) &= \begin{vmatrix} p + k_1 & -A_{12}k_2 & -A_{13}k_3 \\ -A_{21}k_1 & p + k_2 & 0 \\ 0 & -A_{32}k_2 & p + k_3 \end{vmatrix} \\ &= p^3 + (k_1 + k_2 + k_3)p^2 + (k_1k_2 + k_2k_3 + k_3k_1 - k_1k_2A_{12}A_{21})p \\ &\quad + k_1k_2k_3(1 - A_{13}A_{21}A_{32} - A_{12}A_{21}) = 0. \end{aligned}$$

The numerical values of  $s_1$ ,  $s_2$ ,  $s_3$  as well as those of  $k_1$ ,  $k_2$  and  $k_3$  are the same

as before, except that  $k_2 > k_3$ , since the substitution of  $k_2 = 0.03732$  and  $k_3 = 0.04774$  into the equation

$$s_2 = k_1 k_2 + k_2 k_3 + k_3 k_1 - k_1 k_2 A_{12} A_{21} = 0.6696 \times 10^{-3}$$

results in a physically impossible value of greater than one for the constant  $A_{12} A_{21}$ . The tracer distribution curve in lymph pool or  $Q_2$  follows from

$$N_2(p) = \begin{vmatrix} p + k_1 & q_0 & -A_{13}k_3 \\ -A_{21}k_1 & 0 & 0 \\ 0 & 0 & p + k_3 \end{vmatrix},$$

and, except for a small numerical difference in the value of  $A_{21}$  (II), is identical with that for the model I;

$$\frac{q_2}{q_0}(\text{II}) = A_{21}(\text{II})k_1 \left[ -\frac{(\lambda_1 - k_3)e^{-\lambda_1 t}}{(\lambda_1 - \lambda_2)(\lambda_1 - \lambda_3)} + \frac{(\lambda_1 - k_3)e^{-\lambda_2 t}}{(\lambda_1 - \lambda_2)(\lambda_2 - \lambda_3)} + \frac{(k_3 - \lambda_3)e^{-\lambda_3 t}}{(\lambda_1 - \lambda_3)(\lambda_2 - \lambda_3)} \right].$$

$A_{01}$  also remains unchanged, and we get:

$$A_{01} = 0.05328$$

$$A_{12} A_{21} = \frac{k_1 k_2 + k_2 k_3 + k_3 k_1 - s_2}{k_1 k_2} = 0.84941$$

$$A_{13} A_{21} A_{32} = A_{21} A_{32} = 1 - A_{12} A_{21} - \frac{s_3}{k_1 k_2 k_3} = 0.09730$$

$$A_{21} = A_{12} A_{21} + A_{21} A_{32} = 1 - \frac{s_3}{k_1 k_2 k_3} = 0.94672$$

$$A_{12} = \frac{A_{12} A_{21}}{A_{21}} = 0.89722$$

$$A_{32} = 1 - A_{12} = 0.10278$$

Using the same assumed values for  $E_1$ ,  $S_1$  and  $S_3$  as in model I, the pool sizes become:

$$Q_1 = \frac{E_1}{k_1 A_{01}} = 79.4 \mu\text{g (as before)}$$

$$Q_2 = \frac{k_1}{k_2} A_{21} Q_1 = 70.8 \mu\text{g}$$

$$Q_3 = \frac{k_1}{k_3} A_{21} A_{32} Q_1 + \frac{S_3}{k_3} = 102.4 \mu\text{g}$$

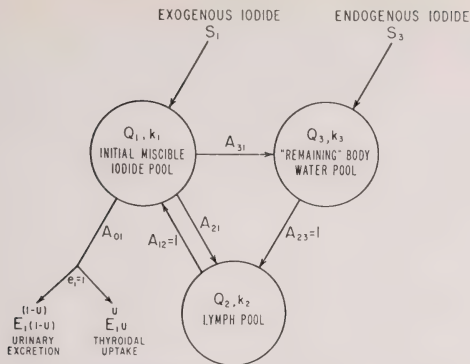


FIG. 3. Schematic representation of iodide metabolism Model III.

*Iodide Model III (Fig. 3).*

This model differs from model II insofar as the unidirectional transfer takes place in the opposite direction, namely from  $Q_1$  to  $Q_3$  to  $Q_2$ . Here  $A_{13} = A_{32} = 0$  and  $A_{23} = A_{12} = 1$

$$\begin{aligned}
 D(p) &= \begin{vmatrix} p + k_1 & A_{12}k_2 & 0 \\ -A_{12}k_1 & p + k_2 & -A_{23}k_3 \\ -A_{13}k_3 & 0 & p + k_3 \end{vmatrix} \\
 &= p^3 + (k_1 + k_2 + k_3)p^2 \\
 &\quad + (k_1k_2 + k_2k_3 + k_3k_1 - A_{21}k_1k_2)p \\
 &\quad + k_1k_2k_3(1 - A_{12}A_{23}A_{31} - A_{12}A_{21}) = 0 \\
 N_2(p) &= \begin{vmatrix} p + k_1 & q_0 & 0 \\ -A_{21}k_1 & 0 & -A_{23}k_3 \\ -A_{13}k_3 & 0 & p + k_3 \end{vmatrix} \\
 &= q_0[A_{21}k_1(p + k_3) + A_{23}A_{31}k_1k_3]
 \end{aligned}$$

and

$$\begin{aligned}
 \frac{q_2}{q_0} &= \frac{A_{21}k_1(k_3 - \lambda_1) + A_{31}k_1k_3}{(\lambda_1 - \lambda_2)(\lambda_1 - \lambda_3)} e^{-\lambda_1 t} \\
 &\quad - \frac{A_{21}k_1(k_3 - \lambda_2) + A_{31}k_1k_3}{(\lambda_1 - \lambda_2)(\lambda_2 - \lambda_3)} e^{-\lambda_2 t} + \frac{A_{21}k_1(k_3 - \lambda_3) + A_{31}k_1k_3}{(\lambda_1 - \lambda_3)(\lambda_2 - \lambda_3)} e^{-\lambda_3 t}.
 \end{aligned}$$



The numerical values of  $s_1$ ,  $s_2$ ,  $s_3$ ,  $k_1$ ,  $k_2$ ,  $k_3$  and  $Q_1$  are again the same as for Models I and II, with  $k_2 > k_3$ . Since  $A_{23} = A_{12} = 1$  and  $A_{03} = A_{02} = 0$ :

$$A_{01} = 0.05328$$

$$A_{21} = A_{12}A_{21} = \frac{k_1k_2 + k_2k_3 + k_3k_1 - s_2}{k_1k_2} = 0.84941$$

$$A_{31} = A_{12}A_{23}A_{31} = 1 - A_{21} - \frac{s_3}{k_1k_2k_3} = 0.09730$$

$$Q_1 = 79.4 \mu g$$

$$Q_2 = \frac{k_1}{k_2} Q_1 - \frac{S_1}{k_2} = 71.5 \mu g$$

$$Q_3 = \frac{k_1}{k_3} Q_1 + \frac{S_3}{k_3} = 102.4 \mu g.$$

Note that here  $Q_3$  is identically equal to the corresponding pool of Model II, i.e.,  $Q_3(\text{II}) = Q_3(\text{III})$ . The constants of the approximating equation  $q_1/q_0 = a_1e^{-\lambda_1 t} + b_1e^{-\lambda_2 t} + c_1e^{-\lambda_3 t}$ , the constants  $k_1$ ,  $k_2$  and  $k_3$ , as well as the pool sizes corresponding to different pool models, are shown for six different subjects (including B.B.) in Table II. The pool sizes were calculated on the basis of the arbitrary assumption that the daily average amounts of iodide released from the degraded hormone and excreted as thyroxine are  $50 \mu g$  and  $7.5 \mu g$ , respectively, for all subjects considered. These values were based on the data of Ingbar and Freinkel (40). As previously noted, doubling the values for hormone degradation and excretion would result in pool sizes twice as large as the ones shown in Table II. In addition, it is highly improbable that the degradation and excretion values of the hormone would be the same (i.e.,  $50 \mu g$  and  $7.5 \mu g$ , respectively), or even in the same ratio (50:7.5), for each subject whose data were analyzed. Therefore, the comparison of relative sizes of various pools should be more meaningful at this point than that of their absolute values. For instance, in the first five subjects considered in Table II, the third pool,  $Q_3$ , is approximately of the same magnitude or larger than the first pool,  $Q_1$ ; only in the sixth subject, P.Z., is  $Q_3$  considerably smaller than  $Q_1$ . The most striking feature of Table II, is perhaps the fact that the respective values for  $Q_2$  and  $Q_3$  of each subject do not vary significantly with the different models considered. Such variations are however most pronounced for  $Q_2$  of subject D.W., where they still do not exceed 22 per cent of the mean value for the three models used. This apparent lack of variation in pool sizes with different three-pool models is not a feature unique to iodide metabolism, but occurs also in three-pool analysis of extrathyroidal thyroxine metabolism (89) and applies to the marrow iron transit pool of the initial three-pool phase of iron metabolism (94). Since the above models used in studying the iodide metabolism represent what may be termed a series of "extreme" three-pool models, any other such models with  $k_2 > k_3$  and the same modes of exit and entry should result in similar pool sizes. As an example, we will now

This table consists of constants of the approximating equation

$$\frac{q_1}{q_0} = a_1 e^{-\lambda_1 t} + a_2 e^{-\lambda_2 t} + a_3 e^{-\lambda_3 t}$$

(*t* in minutes), the calculated relative-rate constants  $k_1$ ,  $k_2$ , and  $k_3$  (relative turnover rates per minute), the calculated fraction,  $1 - u$ , of the injected radio-activity excreted with urine, and finally the calculated pool sizes for the first three models considered above and based on the assumption that 50  $\mu\text{g}$  of iodide,  $S_3$ , are released daily from the degraded thyroxine into the "remaining" body water pool,  $Q_3$ , and 7.5  $\mu\text{g}$  are excreted daily with feces as thyroxine or thyroxine derivatives. In reference to the above models, this is equivalent to the following conditions:  $S_3 = 50 \mu\text{g/day}$ ;  $E_1$  (total amount of iodide leaving the three-pool system) = (57.5/*u*)  $\mu\text{g/day}$  and  $S_1$  (total ingested iodide) = ( $E_1 - S_3$ )  $\mu\text{g/day}$ .

Subject	Diagnosis	Constants of the normalized approximating equation $a_1 e^{-\lambda_1 t} + a_2 e^{-\lambda_2 t} + a_3 e^{-\lambda_3 t}$ ( <i>t</i> in minutes)	Relative rate constants (per minute)	Fraction of initial activity excreted with urine ( $1 - u$ )	$S_3$ Amount (assumed) of iodide released daily from degraded thyroxine into the "remaining" body water pool $Q_3$ (in $\mu\text{g}$ )	$E_1$ Total amount of iodide leaving the three-pool system per day (in $\mu\text{g/day}$ )	$S_1$ Total (daily) injected iodide entering the system through pool $Q_1$ (in $\mu\text{g/day}$ )	$Q_1(I) = Q_2(II) = Q_3(III) = Q_0$ Initial miscible iodide pool (in $\mu\text{g}$ )	$Q_2$ Lymph iodide pool in $\mu\text{g}$	"Remaining" iodide body water pool (in $\mu\text{g}$ )
D.B.	Bilateral inguinal hernia	$a_1 = 0.5814$ $b_1 = 0.1512$ $c_1 = 0.2674$ $\lambda_1 = 0.239$ $\lambda_2 = 0.0230$ $\lambda_3 = 0.00159$	$k_1 = 0.1429$ $k_2 = 0.1061$ $k_3 = 0.01459$	0.84	50	359	309	$Q_1 = 44.2$	$Q_2(I) = 49.4$ $Q_2(II) = 57.1$ $Q_2(III) = 57.4$	$Q_3(I) = 58.0$ $Q_3(II) = 50.3$ $Q_3(III) = 50.3$
B.B.	Prostatic carcinoma gastric ulcer (benign) previously resected	$a_1 = 0.4845$ $b_1 = 0.2494$ $c_1 = 0.2661$ $\lambda_1 = 0.089$ $\lambda_2 = 0.00676$ $\lambda_3 = 0.00071$	$k_1 = 0.04500$ $k_2 = 0.04774$ $k_3 = 0.003732$	0.79	50	274	224	$Q_1 = 79.4$	$Q_2(I) = 62.9$ $Q_2(II) = 70.8$ $Q_2(III) = 71.5$	$Q_3(I) = 110.3$ $Q_3(II) = 102.4$ $Q_3(III) = 102.4$
P.R.	Recurrent inguinal hernia	$a_1 = 0.496$ $b_1 = 0.202$ $c_1 = 0.302$ $\lambda_1 = 0.235$ $\lambda_2 = 0.0244$ $\lambda_3 = 0.0026$	$k_1 = 0.1223$ $k_2 = 0.1243$ $k_3 = 0.01514$	0.741	50	222	172	$Q_1 = 19.6$	$Q_2(I) = 15.6$ $Q_2(II) = 18.0$ $Q_2(III) = 18.2$	$Q_3(I) = 22.2$ $Q_3(II) = 19.8$ $Q_3(III) = 19.8$
D.W.	Gastric ulcer (benign)	$a_1 = 0.551$ $b_1 = 0.234$ $c_1 = 0.215$ $\lambda_1 = 0.278$ $\lambda_2 = 0.0475$ $\lambda_3 = 0.001284$	$k_1 = 0.1632$ $k_2 = 0.1390$ $k_3 = 0.01875$	0.67	50	174	124	$Q_1 = 21.1$	$Q_2(I) = 16.9$ $Q_2(II) = 23.9$ $Q_2(III) = 24.2$	$Q_3(I) = 53.9$ $Q_3(II) = 46.9$ $Q_3(III) = 46.9$
V.S.	Perineal Abscess	$a_1 = 0.429$ $b_1 = 0.311$ $c_1 = 0.260$ $\lambda_1 = 0.210$ $\lambda_2 = 0.025$ $\lambda_3 = 0.0018$	$k_1 = 0.09833$ $k_2 = 0.1266$ $k_3 = 0.01183$	0.664	50	160	110	$Q_1 = 17.7$	$Q_2(I) = 10.1$ $Q_2(II) = 12.8$ $Q_2(III) = 13.1$	$Q_3(I) = 31.6$ $Q_3(II) = 28.9$ $Q_3(III) = 28.9$
P.Z.	Schistosomiasis	$a_1 = 0.5128$ $b_1 = 0.0769$ $c_1 = 0.4103$ $\lambda_1 = 0.116$ $\lambda_2 = 0.0137$ $\lambda_3 = 0.0023$	$k_1 = 0.00148$ $k_2 = 0.05881$ $k_3 = 0.01171$	0.539	50	124.7	74.7	$Q_1 = 16.3$	$Q_2(I) = 14.6$ $Q_2(II) = 15.6$ $Q_2(III) = 17.9$	$Q_3(I) = 7.9$ $Q_3(II) = 6.9$ $Q_3(III) = 6.9$

analyze the experimental data of subject B.B. in terms of a more general three-pool model, IV (Fig. 4). In this model, all interchange constants ( $A_{12}$ ,  $A_{21}$ ,  $A_{23}$ ,  $A_{32}$ ,  $A_{13}$  and  $A_{31}$ ) are non-zero and  $A_{23}$  and  $A_{32}$  are assigned arbitrary values  $a_{23}$  and  $a_{32}$ , respectively.\* Since in previous models (Models I, II, and III), these values varied from zero to 1.0 and from zero to 0.103, respectively, the actual numerical values chosen are  $a_{23} = 0.5$  and  $a_{32} = 0.05$ . The numerical values for  $k_2$  and  $k_3$  of this new model differ slightly from those of models I, II

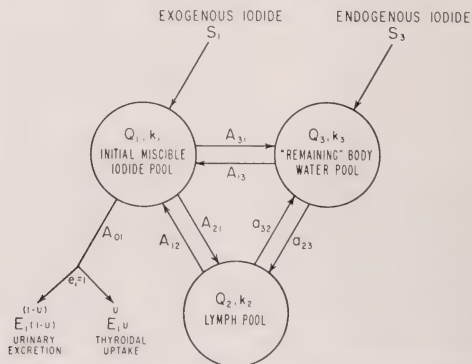


Fig. 4. Schematic representation of iodide metabolism Model IV.

and III (for the derivation of pertinent formulae see the fourth paper of this series) (90):

$$k_2 + k_3 = 0.051475 = X_1 \quad (\text{as in previous models})$$

$$k_2 k_3 = \frac{X_2}{1 - A_{23} A_{32}} = \frac{X_2}{1 - a_{23} a_{32}} = \frac{0.00017818}{0.975} = 0.00018275.$$

Hence, proceeding as before:

$$k_1 = 0.044995$$

$$k_2 = 0.047638$$

$$k_3 = 0.0038362$$

$$Y = \frac{s_3}{k_1 k_2 k_3} = 0.051948$$

$$A_{01} = \frac{Y}{1 - a_{23} a_{32}} = 0.05328$$

\* These values must, however, be consistent with the constants of the approximating equation and the assumed modes of exit and entry.

$$A_{12} = 1 - a_{32} = 0.95000$$

$$A_{13} = 1 - a_{23} = 0.50000$$

$$A_{21} = \frac{k_1 k_2 + k_1 k_3 [a_{23} + (1 - a_{23}) A_{01}] - s_2 + X_2}{k_1 [k_2 (1 - a_{32}) - k_3 (1 - a_{23})]} = 0.89381$$

$$A_{31} = 1 - A_{21} - A_{01} = 0.05291$$

$$Q_1 = 79.4 \mu\text{g} \quad (\text{as for other models}),$$

and finally

$$Q_2 = \frac{k_1 Q_1 (A_{21} + a_{23} A_{31}) + a_{23} S_3}{k_2 (1 - a_{23} a_{32})} = 71.1 \mu\text{g}$$

$$Q_3 = \frac{k_2 Q_2 - A_{21} k_1 Q_1}{a_{23} k_3} = 102.5 \mu\text{g}.$$

These values for  $Q_2$  and  $Q_3$  fall within the respective limits defined by models I, II and III. The summary of all pool constants, calculated on the basis of models I, II, III and IV, respectively, is given, for subject B.B., in Table III.

In the literature, (3a, 5, 47, 79) it is customary to evaluate the total volume of distribution of a metabolite from the extrapolation of the final or "equilibrated" portion of the corresponding plasma disappearance curve. In case of a three-pool system with the given  $Q_1$  and the coefficients of the normalized approximating equation,  $a_1 + b_1 + c_1 = 1$ , this leads to  $Q_1 + Q_2 + Q_3 = Q_1 c_1$ . If, as in the case of iodide subsystem, both  $k_1$  and  $k_2$  are considerably larger than  $k_3$  ( $k_1, k_2 \gg k_3$ ), the remaining pool sizes  $Q_2$  and  $Q_3$  are calculated from  $Q_1$ ,

TABLE III

*Comparison of the Calculated Pool Constants of the Subject B.B., Corresponding to the Four Three-pool Models (Figs. 1, 2, 3 and 4) of Iodide Metabolism*

The approximating equation is

$$\frac{q_1}{q_0} = 0.484e^{-0.089t} + 0.2494e^{-0.0067t} + 0.2661e^{-0.00071t}$$

The following constants are assumed for all four models:

$$E_1 = 0.1903 \mu\text{g/min}$$

$$S_1 = 0.0347 \mu\text{g/min}$$

$$S_3 = 0.1556 \mu\text{g/min}$$

Model	$k_1$	$k_2$	$k_3$	$A_{01}$	$A_{12}$	$A_{21}$	$A_{23}$	$A_{32}$	$A_{14}$	$A_{31}$	$Q_1$	$Q_2$	$Q_3$
I	0.04500	0.04774	0.003732	0.04201	1.00000	0.84116	0.00000*	0.00000*	1.00000	0.10555	79.4	62.9	110.3
II	0.04500	0.04774	0.003732	0.04201	0.89722	0.94672	0.00000*	0.10278	1.00000	0.00000*	79.4	70.8	102.4
III	0.04500	0.04774	0.003732	0.04201	1.00000	0.84941	1.00000	0.00000*	0.00000*	0.09730	79.4	71.5	102.4
IV	0.04500	0.04764	0.003836	0.05328	0.95000	0.89381	0.50000*	0.05000*	0.50000	0.05291	79.4	71.1	102.5

\* The stars refer to the limiting conditions imposed upon two of the interchange constants of each model.

TABLE IV

*Comparison of Various Pool Sizes on Six Euthyroid Subjects, Based on Calculations for Models I, II and III of Iodide Metabolism and on "Extrapolations" from the Approximating Curves to the Plasma  $I^{131}$  Disappearance Data*

Note that the pool sizes obtained from the "extrapolations" are not valid mathematically. See text and next paper in this series (89).

Subject	Method of calculation	$Q_1$ Initial miscible iodide pool in $\mu\text{g}$	$Q_2$ Lymph iodide pool in $\mu\text{g}$	$Q_3$ Remaining body water iodide pool in $\mu\text{g}$	$Q_1 + Q_2 + Q_3$ in $\mu\text{g}$
D.B.	Model I	44.2	49.4	58.0	151.6
	Model II	44.2	57.1	50.3	151.6
	Model III	44.2	57.4	50.3	151.9
	"Extrapolation" from the curve	44.2	61.1	63.8	169.1
B.B.	Model I	79.4	62.9	110.3	252.6
	Model II	79.4	70.8	102.4	252.6
	Model III	79.4	71.5	102.4	253.3
	"Extrapolation" from the curve	79.4	74.6	144.4	298.4
P.R.	Model I	19.6	15.6	22.2	57.4
	Model II	19.6	18.0	19.8	57.4
	Model III	19.6	18.2	19.8	57.6
	"Extrapolation" from the curve	19.6	21.3	26.0	64.9
D.W.	Model I	21.1	16.9	53.9	91.9
	Model II	21.1	23.9	46.9	91.9
	Model III	21.1	24.2	46.9	92.2
	"Extrapolation" from the curve	21.1	25.9	51.1	98.1
V.S.	Model I	17.7	10.1	31.6	59.4
	Model II	17.7	12.8	28.9	59.4
	Model III	17.7	13.1	28.9	59.7
	"Extrapolation" from the curve	17.7	13.4	37.0	68.1
P.Z.	Model I	16.3	14.6	7.9	38.8
	Model II	16.3	15.6	6.9	38.8
	Model III	16.3	17.9	6.9	41.1
	"Extrapolation" from the curve	16.3	17.2	6.3	39.7

$Q_1 + Q_2 + Q_3 = Q_1/c_1$  and  $Q_1 + Q_2 = Q_1/(b_1 + c_1)$  as follows:

$$Q_2 = \frac{a_1}{b_1 + c_1} Q_1; \quad Q_3 = \frac{b_1}{c_1(b_1 + c_1)} Q_1.$$

This method of evaluation of pool sizes is, however, not mathematically valid. Since, in spite of this fact, it is widely used in medical research, it will be elaborated upon in greater detail in the following paper (89).

Table IV gives a summary of various pool sizes corresponding to models I, II and III of iodide metabolism and to the "extrapolations" from the approximating curves to plasma  $I^{131}$  disappearance data, on six euthyroid subjects

In all models considered in this paper, pools  $Q_2$  and  $Q_3$  were tentatively identified with iodide in lymph and in the "remaining" body water, respectively. Such

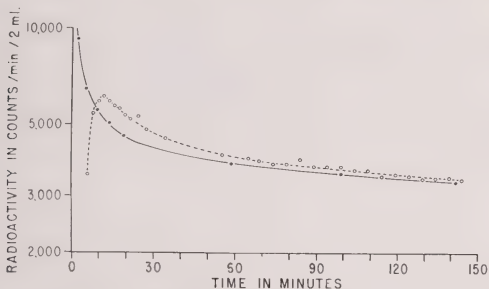


FIG. 5. Radioactivity in plasma and lymph of subject R.P., after an intravenous injection of  $I^{131}$  as iodide, following the thoracic duct cannulation.

●—●—● Radioactivity in plasma  
○- - -○- -○ Radioactivity in lymph

assumptions find considerable substantiation in comparisons of theoretically predicted tracer behavior in lymph with actual lymph radioactivity in human subjects following intravenous injections of  $I^{131}$ , as well as in semiquantitative studies of *in vivo* activity over various body sites following such injections. Figures 5 and 6\* represent simultaneous plasma and lymph activities in subjects R.B. and E.G., respectively, after instantaneous injection of  $I^{131}$  following thoracic duct cannulation in the neck according to the method of Linder and Blomstrand (16, 50). Figure 7 represents normalized theoretical plasma  $I^{131}$  disappearance data

$$\frac{q_1}{q_0} = 0.496e^{-0.235t} + 0.202e^{-0.0244t} + 0.302e^{-0.0025t}$$

on the subject P.R. with the corresponding theoretical lymph radioactivities

\* In Fig. 6, the double peak in the lymph activity and the rise in initial plasma activity probably represent a small subcutaneous leakage of tracer.

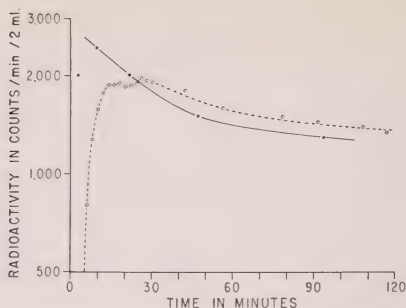


FIG. 6. Radioactivity in plasma and lymph of subject E.G., after an intravenous injection of  $I^{131}$  as iodide, following the thoracic duct cannulation.

•—•—• Radioactivity in plasma  
○—○—○ Radioactivity in lymph

Note that the double peak in the lymph activity and the rise in initial plasma activity represent a probable small subcutaneous leakage of tracer.

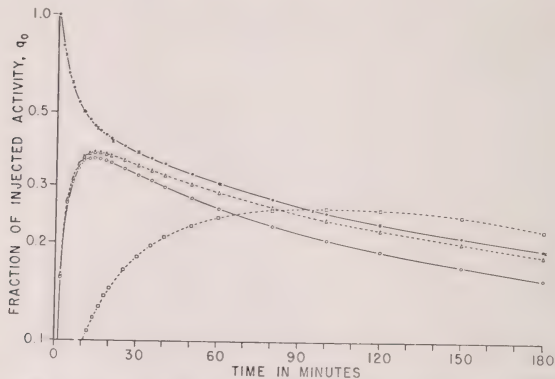


FIG. 7. Theoretical tracer distribution curves (corresponding to various models) of the initial miscible iodide pool,  $Q_1$ , lymph pool,  $Q_2$ , and the "remaining" body water pool,  $Q_3$ , of subject P.R., following an intravenous injection of  $I^{131}$  as iodide.

•—•—•  $(q_1/q_0)$  = normalized plasma  $I^{131}$  disappearance curve (calculated).  
○ ○ ○  $(q_2/q_0)$  (I) = tracer distribution in lymph pool,  $Q_2$ , for model I with  $k_2 > k_3$  (the curve  $(q_2/q_0)$  (II) differs from  $(q_2/q_0)$  (I) by a constant only).  
△—△—△  $(q_2/q_0)$  (III) = tracer distribution in lymph pool,  $Q_2$ , for model III.  
□—□—□  $(q_3/q_0)$  (I) = tracer distribution in the "remaining" body water pool,  $Q_3$ , for model I with  $k_2 > k_3$  (this equals to  $(q_2/q_0)$  (I) with  $k_3 > k_2$ ).

× × × ×

Normalized experimental plasma  $I^{131}$  disappearance data. In the experiments (Figures 5 and 6) lymph was not recirculated. This resulted in a loss of radioiodide from pool II. This loss, however, is not considered in the above models (calculations).



$q_2, q_0$ , for various models considered. Since  $(q_2/q_0)$  (I) with  $k_3 > k_2$  and  $(q_3, q_0)$  (II) differ from each other by a constant factor  $[A_{21}(\text{II})] \cdot [A_{21}(\text{I})]$ , only the lymph curve for model I is represented. Both lymph curves for model I and model III reach a maximum after about 14 minutes and run fairly parallel (on the semilog paper) to the plasma curve after 30 minutes. These curves are similar to the lymph curve in Figure 5. The difference in relative positions or magnitudes between plasma and lymph curves in Figures 5 (or 6) and 7 follows from the fact that while the first set of curves represents actual activities per unit time and unit volume, the second set represents the fractions of the initially injected

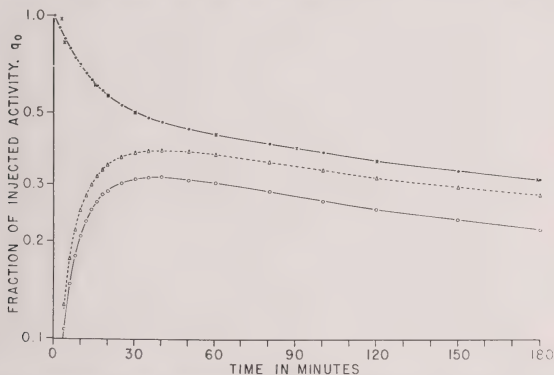


FIG. 8. Theoretical tracer distribution curves (corresponding to different models) of the initial miscible iodide pool,  $Q_1$ , and lymph pool,  $Q_2$ , of subject B.B., after an intravenous injection of  $\text{I}^{131}$  as iodide.

•—•—•  $(q_1/q_0)$  = normalized  $\text{I}^{131}$  disappearance curve in pool  $Q_1$  (calculated).

○—○—○  $(q_2/q_0)$  (I) = tracer distribution in lymph pool,  $Q_2$ , for model I with  $k_2 > k_3$  (the curve  $(q_2/q_0)$  (II) differs from  $(q_2/q_0)$  (I) by a constant only).

Δ—Δ—Δ  $(q_2/q_0)$  (III) = tracer distribution in lymph pool,  $Q_2$ , for model III.

× × × × Normalized experimental plasma  $\text{I}^{131}$  disappearance data.

activity present in the total "initial miscible iodide" and "lymph iodide" pools, respectively. The distribution curve  $q_2, q_0$  for model I with  $k_3 > k_2$  achieves a maximum after about 100 minutes. The two pools, however, are not completely equilibrated, (i.e., their curves are not parallel on semilog paper), for as long as three hours. The curve  $(q_2, q_0)$  (I) for  $k_3 > k_2$  is identical with  $(q_3, q_0)$  (I) for  $k_2 > k_3$ , and serves as an additional indication that  $k_2$  must be larger than  $k_3$ ,  $k_2 > k_3$ , if  $Q_2$  corresponds to the lymph pool. The time of maximum activity in lymph seems to be of the same order of magnitude in human subjects as in dogs. In seven such successful experiments on dogs (87), the times of maximum activity in lymph were approximately 12, 13, 10, 30, 9, 13 and 12 minutes. From theoretical considerations, the time of maximum activity in lymph must increase

with the decrease of the constant  $\lambda_1$ . This dependence is illustrated in Figure 8 which represents the theoretical normalized plasma disappearance curve,  $q_1/q_0$ , as well as the theoretical lymph curves for models I and III on subject B.B. who has the smallest  $\lambda_1$  of all the cases considered. Here the maximum radioactivity is reached only after 40 minutes. This time interval exceeds that in the case of R.P. in Figure 6. In comparing theoretical values with the experimental values in Figures 5 and 6, one should bear in mind that the latter data were collected under conditions where the iodide subsystem was artificially altered by constant drainage of iodide with the continuous lymph flow. This, and the patients'

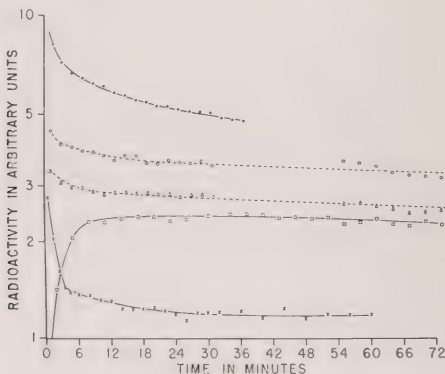


FIG. 9. In vivo activity over various body sites, after an intravenous injection of  $I^{131}$  as iodide.

- Radioactivity over the heart area.
- Radioactivity over the liver area.
- △—△—△ Radioactivity over the splenic area.
- Radioactivity over the calf area.
- ×—×—× Radioactivity over the thyroid gland of another subject who received 0.9 gm of potassium thiocyanate one hour prior to  $I^{131}$  injection.

Note that the radioactivity over each area is expressed in arbitrary and different units to permit convenient spacing of the curves.

postoperative state were among the factors which prevented us from continuing the experiments long enough to allow a mathematical analysis of the actual data. It was noted above that the theoretical activity in the "remaining" body water iodide pool for model I of subject P.R. does not achieve an equilibrium with plasma prior to three hours after injection, i.e., the activities in the respective pools do not form a constant ratio. The same is true of the  $q_3/q_0$  values in the case of the other two models. This agrees well with the general concept that "iodide distribution space" (6) increases over the period of several hours. The hypothesis that  $Q_3$  represents the "remaining" body water iodide pool is further substantiated by the study of in vivo curves over various body sites. Figure 9 represents such typical studies. All values in Figure 9 are plotted on

arbitrary (semilogarithmic) scales to permit convenient visual separation and comparison of results. The counts over the neck were performed on a subject whose thyroid gland was blocked with potassium thiocyanate prior to  $I^{131}$  injection. In trying to interpret these results, one should remember that the counter "sees" all three iodide compartments simultaneously. The actual proportion of activity which different pools contribute to total radioactivity varies with time and location. Thus, when the counter is positioned over the heart, the major contribution, even after the initial surge of activity, is due to  $q_1$ , and the counts fall off fairly rapidly. Counts over spleen, and especially over liver, fall off at the beginning and then decrease very slowly, in spite of the fact that the counter must register a large proportion of blood whose activity is decreasing constantly. The activity over the calf, on the other hand, increases considerably during the first 10-15 minutes, after which it remains almost constant over the period of an hour or two, while that over the blocked gland decreases at the very beginning, and then remains almost constant for the next couple of hours (the particular case used in Figure 9 was not carried out that far). This difference in the behavior between the *in vivo* activity over the neck with the blocked gland and the calf (or a thigh), makes the use of a thigh for correction in counting and estimating the activity in the thyroid gland, rather inadequate.

#### SUMMARY

In the preceding, we have indicated how the iodide component of iodine metabolism can be studied by means of the mathematical analysis of plasma  $I^{131}$  disappearance data and urinary excretion, based on the assumption of three-pool systems. These pools can be identified as

- a) Initial miscible iodide pool consisting primarily of iodide in plasma, red cells, gastric mucosa and salivary glands, and other presumed iodide concentrating tissues (including the contribution of thyroïdal trapping).
- b) Iodide in "central lymph" and possibly in peripheral lymph beds.
- c) Iodide in the "remaining" body water.

Several tentative three-pool models have been developed in detail and the results of their applications to experimental plasma  $I^{131}$  data compared with tracer distribution data in lymph and in a semiquantitative way with the tracer activity over various body sites as detected by *in vivo* counting. The actual "average" sizes of the pools corresponding to various models can be determined only if simultaneous data about the rate of thyroxine degradation and excretion are available. The dynamics of thyroxine metabolism and the possibility of simultaneous study of inorganic and organic components of the iodine metabolism will form the subject of the following paper.

## Studies in Iodine Metabolism

### III. Three-Pool Systems of Extrathyroidal Thyroxine Kinetics\*

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Whereas the iodide subsystem of iodine metabolism is a first order non-steady-state system, the organic iodine subsystem is usually in a dynamic equilibrium. The rate of its hormone degradation is assumed to be non-linear (79) and according to Berson and Yalow (5), is directly proportional to the square of the thyroxine level in the plasma, at least when this level is within the thyrotoxic region. (Two of their cases, whose plasma thyroxine concentration falls into the euthyroid category, appear to give equivocal results.) When a metabolic system is in dynamic equilibrium, its general behavior, as reflected in the changes in radioactive tracers, is indistinguishable from that of a first order system, regardless of the actual order of its various reaction rates and processes. A series of plasma thyroxine disappearance experiments (using exogenous  $I^{131}$  labelled sodium-1-thyroxine) were performed both by the workers in this country and in England with results that differ significantly from each other. The average degradation rates (half life) of exogenous hormone in euthyroid patients vary from 6.5 (37) to 8.0 days (21). Some of these variations may be attributed to differences in the particular thyroxine preparations administered, choice of subjects, duration of experiments, pretreatment with thyroid-blocking agents such as Lugol's solution, or other experimental manipulations. Many interesting and ingenious methods were developed to determine the amount of the hormone in the thyroid gland (8, 62, 67) and the rate of its peripheral degradation, after a single injection of  $I^{131}$  as iodide (18, 21, 27, 37, 63, 79, 103). In this paper we will be concerned exclusively with the behavior of the exogenously administered  $I^{131}$  labelled hormone. The space in which the exogenous  $I^{131}$  labelled hormone is distributed after an intravenous injection is defined by Riggs as the "Organic Iodine of the Blood and Extrathyroidal Tissue. This compartment contains all of the thyroid hormone outside of the thyroid gland including any thyroxine which is undergoing enterohepatic circulation." Most of the workers in the field agree in considering all of the extrathyroidal thyroxine as a single compartment or "thyroxine distribution space" (37, 79), and attribute errors inherent in the methods used for determination of such a single space, as well as those in calculation of total fecal excretion, to the delay in mixing. The formidable mathematical problems connected with the delayed mixing within individual compartments

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are dealt with by such investigators as Wrenshall et al (113). Such considerations do not apply to pool systems where tracer disappearance curves can be approximated by a sum of a few (three or four) exponential functions with constant coefficients,  $\alpha_1 e^{-\lambda_1 t}$ . We will return again to this so-called "mixing problem" after the formulation of three-pool models of extrathyroidal thyroxine metabolism. The disappearance curves of  $I^{131}$  labelled thyroxine from plasma (after an instantaneous intravenous injection) differ little, except in the magnitudes of their constants, from the corresponding tracer distribution curves of  $Fe^{59}$ -transferrin or of  $I^{131}$  as iodide. These curves, when followed over a period of eight or more days, can be fairly well approximated by sums of three exponential functions (i.e., by  $\alpha_1 e^{-\lambda_1 t} + \alpha_2 e^{-\lambda_2 t} + \alpha_3 e^{-\lambda_3 t}$ ), indicating the existence of at least three (92) closely interchanging extrathyroidal thyroxine pools. The exact modes of connections of these pools do not follow from the above approximating equations, and it will be the purpose of this paper to build and solve several mathematical models of this subsystem of iodine metabolism.\*

We will proceed as in the paper on iodide subsystem's, by using four three-pool models after imposing upon them the necessary number of the simplest possible (at least from the mathematical point of view) conditions, such as setting certain interchange constants,  $A_{ij}$ , equal to zero (92). We will assume that, at least from the point of view of thyroxine dynamics, these three extrathyroidal thyroxine pools consist of (1) plasma pool, or iodine in plasma thyroxine; (2) "intrahepatic" pool, or iodine in intrahepatic thyroxine and other related organic compounds; (3) lymph pool, or iodine in thyroxine (or its derivatives, excluding iodide) of lymph and other interstitial fluids. That plasma forms a single well-delineated pool follows from the fact that post-injection plasma activity, when extrapolated to zero time, leads to the initial thyroxine space which is identical with that of the plasma volume. The identification of one of the other two pools with central lymph (and probably remaining interstitial fluid) follows from the lymph experiments on dogs and humans similar to those performed in the study of iodide metabolism. The result of these experiments will be discussed in detail later in this paper. Justification of the assumption of a separate "liver" pool follows both from the known facts (43, 64, 104) on bile and fecal excretion, and in vivo counting, as well as from our own quantitative observations of the bile activity after intravenous thyroxine  $I^{131}$  injection into several dogs (after transduodenal cannulation of the common bile duct) and into a subject with gangrenous cholecystitis with a common duct drainage tube. These results will also be discussed later. We performed several plasma sodium-1-thyroxine disappearance experiments with  $I^{131}$  labelled thyroxine, which was obtained both in water-soluble

\* The definitions of the terms used and a summary of the fundamental assumptions underlying the formulation of  $n$ -pool models of first order metabolic systems, as well as the actual formulations and solutions of such models are discussed at length in "Multiple-pool analysis in tracer studies of metabolic kinetics" (92) and in "Studies of iodine metabolism: IV" (90). The latter paper deals specifically with three-pool models which are applicable directly to iodide (93) and thyroxine subsystems of iodine metabolism and to the initial three-pool subsystem of iron metabolism (94).



form (Squibb)\* and in 50 per cent propylene glycol (Abbott). All subjects received three doses daily of 5-10 drops of Lugol's solution during the experiment and at least for two days prior to the initial injection of the exogenous  $I^{131}$  labelled sodium-L-thyroxine. Blood specimens were collected at convenient intervals, at first every five minutes, then hourly and finally daily; plasma samples were counted as in the iodide experiment (93). Stool specimens were collected over a period of at least a week or more, and the cumulative activity extrapolated (to its asymptotic maximum) to obtain the total fraction of initial activity eventually excreted as thyroxine or its derivatives in feces. Urines were also collected and counted, though these results are not elaborated upon in the present study.

The main purpose of blocking the thyroid gland with stable iodide during the experimental period is to prevent the reassimilation of  $I^{131}$  by the thyroid. Such recycling of  $I^{131}$  derived from the degradation of labelled hormone can be considerable in cases of thyrotoxicosis (102), and is probably grossly underestimated in the studies of hormone degradation in euthyroid subjects (8, 56). Although it is a known fact that large amounts of potassium iodide inhibit the release of hormone from the thyroid gland of patients with hyperthyroidism (24), we expected to encounter no significant changes in thyroxine metabolism in euthyroid patients during the experimental period of ten to fourteen days with daily intakes of stable  $I^{127}$  not exceeding 200-300 mg. Such a steady state should guarantee a constant rate of degradation of the hormone (even though, as was noted above, this process may not be a first order reaction and may change with the square of the concentration of thyroxine in plasma) and hence a constant rate of  $I^{131}$  disappearance from plasma, after a final equilibrium. In most of our euthyroid subjects, however, the data corresponding to the third exponential components of the plasma  $I^{131}$  thyroxine disappearance curves (which should approximate straight lines on semilogarithmic paper after the second or third day following injection of labelled sodium-L-thyroxine), have exhibited too much variability to be explained on the basis of diurnal variations of plasma volume or other errors inherent in prolonged biological experiments. In some cases, after 4 to 7 days the slope of  $I^{131}$  activity begins definitely to decrease (Fig. 1). This phenomenon is also apparent in the data shown by Goldsmith and Eisele (23). Such variability can be explained on the basis of small changes of thyroxine levels following  $I^{127}$  administration, especially if one remembers that the process of hormone degradation is a second order reaction and, therefore, any changes in radioactivity will appear greatly exaggerated.<sup>†</sup> The above considerations are supported by the work of C. L. Mercer, et al who demonstrated in their work the "slowing of thyroid secretion by iodide in euthyroid people" (56). Similar thyroxine  $I^{131}$  disappearance curves may reflect the repeated reassimilation by the thyroid gland of  $I^{131}$  derived from degraded hormone (40).

It will be suggested at the end of this paper how errors in the estimation of the

\* Generously supplied by The Squibb Institute for Medical Research through the courtesy of Dr. G. E. Lindenblad and Dr. P. Numerof.

<sup>†</sup> The assumption of the existence of a fourth exponential component does not seem to be justified on the basis of available clinical data.

rate of hormone degradation (due either to the blocking of the gland by  $I^{127}$  or the recycling of  $I^{131}$ ) can be avoided or corrected by means of the double isotope technique. In the following text, several three-pool models of extra-thyroidal thyroxine metabolism will be formulated after the outline of the preceding paper on iodide metabolism.

Detailed discussion on methodology, basic assumptions and mathematical treatment of models representing reactions or processes which can be described in terms of linear homogeneous differential equations with constant coefficients can be found in "Multiple-pool analysis in tracer studies of metabolic kinetics" (92). A brief review of definitions, notations, and necessary equations for solving

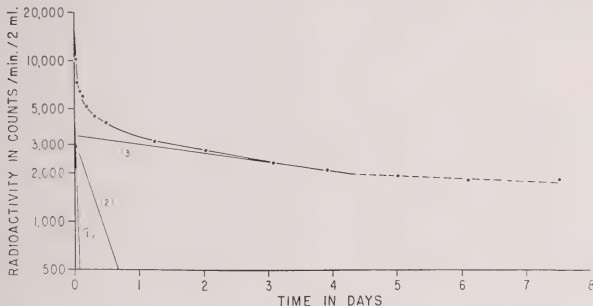


FIG. 1. Plasma thyroxine  $I^{131}$  disappearance data on a euthyroid subject, R.G., receiving daily doses of 300 mg. of  $I^{127}$ . The disappearance curve can be expressed as a sum of three exponential components ( $\alpha_1 e^{-\lambda_1 t} + \alpha_2 e^{-\lambda_2 t} + \alpha_3 e^{-\lambda_3 t}$ ) during the first four days after thyroxine  $I^{131}$  injection, and shows an abrupt change in the slope of the third component ( $\alpha_3 e^{-\lambda_3 t}$ ) after the fourth day.

●●● Experimental plasma thyroxine  $I^{131}$  disappearance data.

- (1) is the first component  $\alpha_1 e^{-\lambda_1 t}$
- (2) is the second component  $\alpha_2 e^{-\lambda_2 t}$
- (3) is the third component  $\alpha_3 e^{-\lambda_3 t}$

three-pool systems are given in the fourth paper of this series (90). The three separate pools were already tentatively identified, and will be referred to as:

- 1)  $Q_1$  = plasma pool consisting of iodine in plasma thyroxine.\*
- 2)  $Q_2$  = "intrahepatic" pool consisting of iodine in intrahepatic thyroxine and other organic compounds.
- 3)  $Q_3$  = lymph pool, consisting of iodine in thyroxine\* of lymph, other interstitial fluids, and remaining body water.

The five alternate models A, B, C, D, and E, are schematically represented in Figures 2, 3, 4, 5, and 6, and were selected according to the principles discussed in the preceding paper. The modes of exit and entry are assumed to be the same for each of the five models considered: It is assumed that  $e_1 = E_1 = 0$ ;  $e_2$  and

\* Here "thyroxine" may refer to at least in part to its derivatives.



$c_i$  are the fractions of initially injected isotope leaving the system with  $E_2$  (fecal excretion) and  $E_3$  (iodide from the degraded thyroxine released into iodide subsystem), respectively. It is further assumed that  $S_2 = S_3 = 0$ , i.e., that all thyroxine enters the extrathyroidal thyroxine subsystem through pool  $Q_1$ . Two additional constraints are imposed upon the system by limiting the numerical values of the interchange constants  $A_{12}$ ,  $A_{21}$ ,  $A_{23}$ ,  $A_{32}$ ,  $A_{13}$  and  $A_{31}$ . It will be shown (90) that under the conditions of all entry through the accessible pool  $Q_1$ , the actual numerical values of  $E_2$  and  $E_3$  are proportional to  $c_2$  and  $c_3$ , respectively, and do not depend upon the values of the interchange constants defining particular three-pool models.

As a numerical example we will use the data on the euthyroid patient J.S.

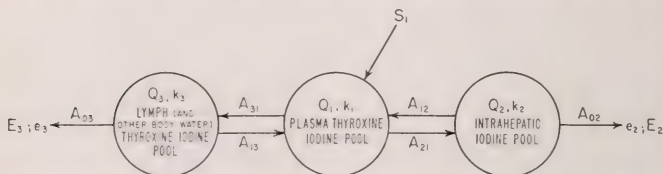


FIG. 2. Schematic representation of Model A of the extrathyroidal thyroxine metabolism.

whose plasma thyroxine  $I^{131}$  disappearance data were approximated by the sum of three exponential functions:

$$\frac{q_1}{q_0} = 0.435e^{-0.02665t} + 0.281e^{-0.001191t} + 0.284e^{-0.00007452t},$$

where  $t$  is in minutes. The total fraction of initially injected isotope which is eventually excreted with feces (determined by an extrapolation method similar to the one described for urine in the preceding paper) is given by  $c_2 = 0.03$ ; hence the fraction of the initially injected isotope which is eventually released through  $E_3$  into the iodide subsystem is equal to  $c_3 = 1 - c_2 = 0.97$ . The thyroxine iodine content of plasma is given by  $Q_1 = 134.3 \mu\text{g}$  (plasma volume in  $\text{ml} \times \mu\text{g}$  of  $\text{PBI}^{127} \text{ ml}$ ). The models A, B, and C, are similar to the models I, II, and III, respectively of the iodide metabolism, except that they have different modes of exit and entry, and that the subscripts 2 and 3 are reversed in the second group of models. Similarly, model D corresponds to one of the models used in "Studies in iron kinetics: IV" (94), and model E (with all interchange constants greater than zero) is discussed in detail in the fourth paper of this series (90). Therefore, for the sake of brevity, the detailed descriptions of individual models, as well as explicit writing of all determinants  $D(p)$  and  $N_i(p)$  will be omitted, and only expressions leading immediately to the solutions of the respective pool systems will be used. The following values are calculated for all four models:

$$s_1 = \lambda_1 + \lambda_2 + \lambda_3 = 0.027916$$

$$s_2 = \lambda_1\lambda_2 + \lambda_2\lambda_3 + \lambda_3\lambda_1 = 0.033815 \times 10^{-3}$$

$$s_3 = \lambda_1\lambda_2\lambda_3 = 0.0023653 \times 10^{-6}$$

$$X_1 = a_1(\lambda_2 + \lambda_3) + b_1(\lambda_1 + \lambda_3) + c_1(\lambda_1 + \lambda_2) = 0.015967$$

$$X_2 = a_1\lambda_2\lambda_3 + b_1\lambda_1\lambda_3 + c_1\lambda_1\lambda_2 = 0.00961086 \times 10^{-3}.$$

$k_1$ ,  $k_2$ , and  $k_3$  are the same for models A, B, and C.  $k_3 > k_2$  is the only combination of  $k_3$  and  $k_2$  that can satisfy models B and C, while in model A,  $k_2 > k_3$  is theoretically possible. (In case of model D,  $k_3$ , and therefore  $k_2$ , has only one solution.)

Setting  $X_1 = k_2 + k_3$  and  $X_2 = k_2k_3$ , the relative disappearance rate constants can be solved for models A, B, and C, following the scheme used for models I, II, and III of iodide metabolism (92, 93):

$$k_1 = 0.01195$$

$$k_2 = 0.0006265$$

$$k_3 = 0.01534.$$

#### Model A:

This model is similar to model I (93). It is assumed that  $A_{23} = A_{32} = 0$ . Also  $e_1 = 0$ ;  $e_2 = 0.03$ ;  $e_3 = 0.97$ .

$$A_{12}A_{21} = \frac{s_3 - k_2s_2 + k_2^2(k_1 + k_3)}{k_1k_2(k_2 - k_3)} = 0.07362$$

$$A_{13}A_{31} = \frac{s_2k_3 - s_3 - k_3^2(k_1 + k_2)}{k_1k_3(k_2 - k_3)} = 0.90579$$

$$A_{21} = A_{12}A_{21} + \frac{e_2s_3}{k_1k_2k_3} = 0.07424$$

$$A_{12} = \frac{A_{12}A_{21}}{A_{21}} = 0.99168$$

$$A_{02} = 1 - A_{12} = 0.00832$$

$$A_{31} = 1 - A_{21} = 0.92576$$

$$A_{03} = \frac{1 - e_2s_3}{k_1k_2k_3A_{31}} = 0.02158$$

$$A_{13} = 1 - A_{03} = 0.97842$$

$$Q_1 = 134.3 \mu g \text{ (given)}$$

$$Q_2 = \frac{k_1}{k_2} A_{21} Q_1 = 190.2 \mu g$$

$$E_2 = k_2 A_{02} Q_2 \times 1440 = 1.43 \mu g/\text{day}$$

$$Q_3 = \frac{k_1}{k_3} A_{31} Q_1 = 96.9 \mu g$$

$$E_{31} = k_3 A_{03} Q_3 \times 1440 = 46.2 \mu g/\text{day}$$

$$\frac{q_2}{q_0} = A_{21} k_1 \left[ -\frac{(\lambda_1 - k_3)e^{-\lambda_1 t}}{(\lambda_1 - \lambda_2)(\lambda_1 - \lambda_3)} + \frac{(\lambda_2 - k_3)e^{-\lambda_2 t}}{(\lambda_1 - \lambda_2)(\lambda_2 - \lambda_3)} \right. \\ \left. + \frac{(k_3 - \lambda_3)e^{-\lambda_3 t}}{(\lambda_1 - \lambda_3)(\lambda_2 - \lambda_3)} \right]$$

$$= -0.0148e^{0.02665t} - 0.4411e^{-0.001191t} + 0.4559e^{-0.0007452t}$$

$$\frac{q_3}{q_0} = A_{31} k_1 = \left[ -\frac{(\lambda_1 - k_2)e^{-\lambda_1 t}}{(\lambda_1 - \lambda_2)(\lambda_1 - \lambda_3)} + \frac{(\lambda_2 - k_2)e^{-\lambda_2 t}}{(\lambda_1 - \lambda_2)(\lambda_2 - \lambda_3)} \right. \\ \left. + \frac{(k_2 - \lambda_3)e^{-\lambda_3 t}}{(\lambda_1 - \lambda_3)(\lambda_2 - \lambda_3)} \right]$$

$$= -0.4251e^{0.02665t} + 0.2195e^{-0.001191t} + 0.2056e^{-0.0007452t}$$

In the above, the factor 1440 was used to change the rate of transfer per minute to rate per day. It should be noted that the values  $E_2$  and  $E_3$  are identical, respectively, for all four models considered and can be shown (90) to be:

$$E_2 = \frac{e_2 s_3 Q_1}{X_2} \quad \text{and} \quad E_3 = \frac{e_3 s_3 Q_1}{X_2}$$

If  $k_2$  is assumed larger than  $k_3$ ,  $E_2$  and  $E_3$  still remain the same while the respective pool sizes become:

$$Q_2 = 94.6 \mu g,$$

and

$$Q_3 = 239.8 \mu g.$$

*Model B:*

This model is similar to model II (93), though here  $A_{32} = A_{21} = 0$  and  $A_{31} = 1$ . The values of  $e_i$  are as before:  $e_1 = 0$ ;  $e_2 = 0.03$ ;  $e_3 = 0.97$ :

$$A_{13} = A_{13} A_{31} = \frac{k_1 k_2 + k_2 k_3 + k_3 k_1 - s_2}{k_1 k_3} = 0.90879$$

$$A_{12} A_{23} = A_{12} A_{31} A_{23} = 1 - A_{13} A_{31} - \frac{s_3}{k_1 k_2 k_3} = 0.07061$$

$$A_{03} = \frac{e_3 s_3}{k_1 k_2 k_3} = 0.01998$$

$$A_{23} = 1 - A_{13} - A_{03} = 0.07123$$

$$A_{12} = \frac{A_{12} A_{23}}{A_{23}} = 0.99133$$

$$A_{02} = 1 - A_{12} = 0.00867$$

$$Q_2 = \frac{k_1}{k_2} A_{23} Q_1 = 182.5 \mu g$$

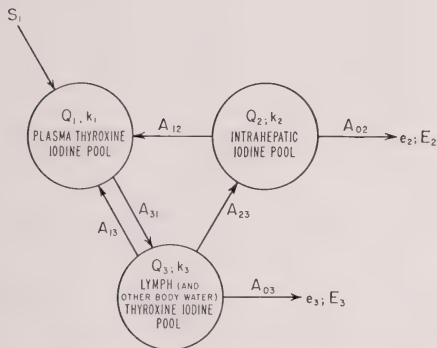


FIG. 3. Schematic representation of Model B of the extrathyroidal thyroxine metabolism.

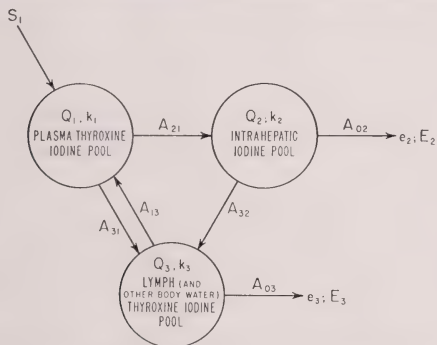


FIG. 4. Schematic representation of Model C of the extrathyroidal thyroxine metabolism.

$$E_2 = 1.43 \mu g \text{ day}$$

$$Q_3 = \frac{k_1}{k_3} Q_1 = 104.6 \mu g$$

$$E_3 = 46.2 \mu g \text{ day}$$

$$\frac{q_3}{q_0} = A_{31}k_1 \left[ -\frac{(\lambda_1 - k_2)e^{-\lambda_1 t}}{(\lambda_1 - \lambda_2)(\lambda_2 - \lambda_3)} + \frac{(\lambda_2 - k_2)e^{-\lambda_2 t}}{(\lambda_1 - \lambda_2)(\lambda_2 - \lambda_3)} \right. \\ \left. + \frac{(k_2 - \lambda_3)e^{-\lambda_3 t}}{(\lambda_1 - \lambda_3)(\lambda_2 - \lambda_3)} \right].$$

*Model C:*

This model is similar to model III (93), though here  $A_{12} = A_{23} = 0$ . The values of  $e_i$  are as before:  $e_1 = 0$ ;  $e_2 = 0.03$ ;  $e_3 = 0.97$ .

$$A_{13}A_{31} = \frac{k_1k_2 + k_2k_3 + k_3k_1 - s_2}{k_1k_3} = 0.90879$$

$$A_{13}A_{32}A_{21} = 1 - A_{13}A_{31} - \frac{s_3}{k_1k_2k_3} = 0.07061$$

$$A_{31} = \frac{A_{13}A_{31}(1 - A_{02}A_{21})}{A_{13}A_{31} + A_{13}A_{32}A_{21}} = \frac{k_1k_2k_3 + k_2^2(k_1 + k_3) - s_2k_2}{k_1k_2k_3 - s_3} \left( 1 - \frac{e_2s_3}{k_1k_2k_3} \right) \\ = 0.92733$$

$$A_{13} = \frac{A_{13}A_{31}}{A_{31}} = \frac{k_1k_2k_3 - s_3}{k_1k_2k_3 - e_2s_3} = 0.98001$$

$$A_{21} = 1 - A_{31} = 0.07267$$

$$A_{02} = \frac{e_2s_3}{k_1k_2k_3A_{21}} = 0.00850$$

$$A_{32} = 1 - A_{02} = 0.99150$$

$$A_{03} = 1 - A_{13} = 0.01999$$

$$Q_2 = \frac{k_1}{k_2} A_{21}Q_1 = 186.2 \mu g$$

$$E_2 = 1.43 \mu g/\text{day}$$

$$Q_3 = \frac{k_1}{k_3} (A_{31} + A_{21}A_{32})Q_1 = 104.6 \mu g$$

$$E_3 = 46.2 \mu g$$

$$\frac{q_3}{q_0} = \frac{A_{31}k_1(k_2 - \lambda_1) + A_{32}A_{21}k_1k_2}{(\lambda_1 - \lambda_2)(\lambda_1 - \lambda_3)} e^{-\lambda_1 t} \\ - \frac{A_{31}k_1(k_2 - \lambda_2) + A_{32}A_{21}k_1k_2}{(\lambda_1 - \lambda_2)(\lambda_2 - \lambda_3)} e^{-\lambda_2 t} \\ + \frac{A_{31}k_1(k_2 - \lambda_3) + A_{32}A_{21}k_1k_2}{(\lambda_1 - \lambda_3)(\lambda_2 - \lambda_3)} e^{-\lambda_3 t}.$$

*Model D:*

In this model  $A_{12} = A_{21} = 0$ ;  $A_{31} = 1$ ;  $e_1 = 0$ ;  $e_2 = 0.03$ ;  $e_3 = 0.97$ . Here  $k_2$  and  $k_3$  differ from the corresponding values in models A, B, and C (92, 94):

$$k_2 = \frac{X_2 s_1 - X_1 X_2 - s_3}{X_1 s_1 + X_2 - X_1^2 - s_2} = 0.00067518$$

$$k_3 = X_1 - k_2 = 0.015292$$

$$k_1 = s_1 - X_1 = 0.01195$$

$$A_{32}A_{23} = \frac{s_2 k_2 - s_3 - k_2^2 (k_1 + k_2)}{k_2 k_3 (k_1 - k_2)} = 0.06914$$

$$A_{13} = A_{13}A_{31} = \frac{s_3 - s_2 k_1 + k_1^2 (k_2 + k_3)}{k_1 k_3 (k_1 - k_2)} = 0.91168$$

$$A_{03} = \frac{e_3 s_3}{k_1 k_2 k_3} = 0.01860$$

$$A_{23} = 1 - A_{13} - A_{03} = 0.06972$$

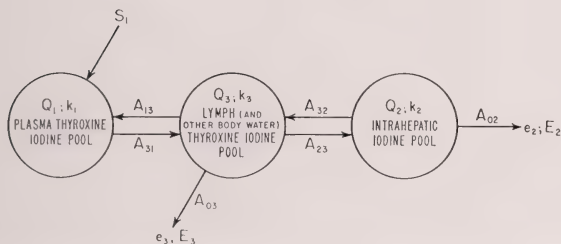


FIG. 5. Schematic representation of Model D of the extrathyroidal thyroxine metabolism.

$$A_{32} = \frac{A_{32}A_{23}}{A_{23}} = 0.99172$$

$$A_{02} = 1 - A_{32} = 0.00828$$

$$Q_2 = \frac{k_1 A_{23} Q_1}{k_2 (1 - A_{23} A_{32})} = 178.0 \mu g$$

$$E_2 = 1.43 \mu g/\text{day}$$

$$Q_3 = \frac{k_1 Q_1}{k_3 (1 - A_{23} A_{32})} = 112.8 \mu g$$

$$E_3 = 46.2 \mu g/\text{day}.$$

Here as in the case of three-pool iodide subsystems, the pool sizes ( $Q_2$  and  $Q_3$ , respectively) do not change significantly with different models, two of whose interchange constants are set equal to zero (see Table I). Following the scheme used in the study of iodide subsystems (93), "average values" are assigned to

TABLE I  
Comparison of the Calculated Pool Constants of the Subjects T.S. and R.D., Corresponding to the Five Three-pool Models (Figs. 2, 3, 4, 5, 6) of the Extrathyroidal Thyroxine Metabolism

Subject	Diagnosis	Constants of the Approximating Equation				$\epsilon_8$	$\epsilon_1$	Model	$k_1$	$k_2$	$k_d$	$A_{ee}$	$A_{e2}$	$A_{17}$	$A_{21}$	$A_{33}$	$A_{37}$	$Q_1$ $\mu\text{g}$	$Q_7$ $\mu\text{g/day}$	$E_7$ $\mu\text{g/day}$	$Q_8$ $\mu\text{g}$	$E_8$ $\mu\text{g/day}$			
J.S.	Allergic asthma, or toxicoderma and cholestatic disease	$a_1 = 0.435$	$\lambda_1 = 0.02665$	$\epsilon = 0.030$	$\epsilon = 0.970$	0.030	0.970	A	0.01195	0.000265	0.01531	0.00832	0.02158	0.99168	0.07121	0*	0.97842	0.92576	134.3	190.2	1.43	96.9	16.2		
		$b_1 = 0.284$	$\lambda_2 = 0.001191$						B	0.01195	0.000265	0.01531	0.00867	0.01998	0.99133	0*	0.07123	0*	0.90879	1.00000	131.3	182.5	1.43	101.6	46.2
		$c_1 = 0.284$	$\lambda_3 = 0.00007452$						C	0.01195	0.000265	0.01531	0.00850	0.01999	0*	0.07267	0*	0.99111	0.98001	0.92733	131.3	186.2	1.43	101.6	46.2
									D	0.01195	0.000752	0.01529	0.00828	0.01860	0*	0.06972	0	0.99472	0.91168	1.00000	131.3	178.0	1.43	112.8	46.2
								E	0.01195	0.000365	0.01533	0.00812	0.02012	0.49158	0.01357	0.03000*	0.50000*	0.91988	0.35613	131.3	185.0	1.43	101.0	46.2	
R.D.**	Lacune's cirrhosis of the liver	$a_1 = 0.3269$	$\lambda_1 = 0.0277$	0.032	0.968	A	0.009601	0.0010353	0.018922	0.00792	0.02741	0.99208	0.10209	0*	0.97259	0.89731	235.2	221.0	2.61	107.1	80.0				
		$b_1 = 0.2885$	$\lambda_2 = 0.00176$						B	0.009601	0.0010353	0.018922	0.00837	0.02450	0.99163	0*	0.00712	0*	0.87820	1.00000	235.2	211.8	2.61	119.3	80.0
		$c_1 = 0.3806$	$\lambda_3 = 0.000098$						C	0.009601	0.0010353	0.018922	0.00817	0.02461	0*	0.09955	0*	0.99183	0.97539	0.90045	235.2	217.1	2.61	119.3	80.0
									D	0.009601	0.001119	0.018809	0.00783	0.02229	0*	0*	0.09111	0.99217	0.88359	1.00000	235.2	204.0	2.61	132.4	80.0
								E	0.009601	0.0010634	0.018894	0.00805	0.02460	0.49195	0.05102	0.05000*	0.50000*	0.92540	0.94808	235.2	211.4	2.61	119.4	80.0	

\* Refers to the limiting conditions imposed upon two of the interchange constants of each model.

\*\* Subject R.D. had 2 cholangiograms within a month prior to the experiment. Since no thyroxine determinations could be done, the PM<sub>177</sub> was assumed to be  $4.1 \mu\text{g}/100 \text{ cc} = 235.2 \mu\text{g}/\text{total plasma volume}$ . In this case, the absolute thyroxine value may be somewhat misleading. It should be noted, however, that for subject R.D.,  $Q_1$  is slightly larger than  $Q_2$  and about twice as large as  $Q_7$ . In the case of subject J.S., however,  $Q_1$  is significantly smaller than  $Q_7$ , and only slightly larger than  $Q_2$ .



constants  $A_{32}$  and  $A_{23}$  and a new model E is formed with none of its six interchange constants equal to zero. This model is discussed at length in part II of "Studies of iodine metabolism. IV" (90), and therefore only final and numerical terms will appear in this text.

#### Model E:

Let  $A_{23} = a_{23} = 0.03$ ;  $A_{32} = a_{32} = 0.5$  and  $e_1 = 0$ ;  $e_2 = 0.03$ ,  $e_3 = 0.97$ . Following the above-mentioned scheme, we get:

$$k_2 + k_3 = 0.015967 = X_1$$

$$k_2 k_3 = \frac{X_2}{1 - a_{23}a_{32}} = 0.00975722 \times 10^{-3}$$

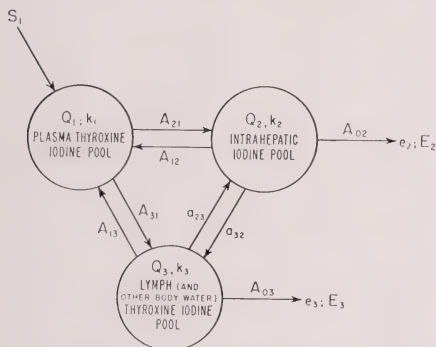


FIG. 6. Schematic representation of Model E of the extrathyroidal thyroxine metabolism.

and therefore

$$k_1 = 0.011949$$

$$k_2 = 0.00063646$$

$$k_3 = 0.015330.$$

Furthermore:

$$Y = \frac{s_3}{k_1 k_2 k_3} = 0.020288.$$

After appropriate substitutions (90) the third degree equation in  $A_{21}$ , becomes:

$$A_{21}^3 - 1.99144A_{21}^2 + 0.0236526A_{21} + 0.00266770 = 0.$$

This equation has three solutions:

$$1) A_{21} = 0.0435736$$

$$2) A_{21} = 1.978806$$

$$3) A_{21} = -0.0030939,$$

only one of which (namely the first) satisfies the general pool conditions. Therefore:

$$A_{21} = 0.04357$$

$$A_{31} = 1 - A_{21} = 0.95643$$

$$A_{02} = \frac{c_2 Y}{a_{23} A_{31} + A_{21}} = 0.00842$$

$$A_{03} = \frac{c_3 Y}{a_{32} A_{21} + A_{31}} = 0.02012$$

$$A_{12} = 1 - A_{02} - a_{32} = 0.49158$$

$$A_{13} = 1 - a_{23} - A_{03} = 0.94988$$

$$Q_1 = 134.3 \text{ } \mu\text{g (given)}$$

$$Q_2 = \frac{k_1}{k_2} Q_1 \frac{A_{21} + a_{23} A_{31}}{1 - a_{23} a_{32}} = 185.0 \text{ } \mu\text{g}$$

$$E_2 = k_2 A_{02} Q_2 = 0.0009917 \text{ } \mu\text{g/min} = 1.43 \text{ } \mu\text{g/day}$$

$$Q_3 = \frac{k_1}{k_3} Q_1 \frac{A_{31} + a_{32} A_{21}}{1 - a_{23} a_{32}} = 104.0 \text{ } \mu\text{g}$$

$$E_3 = k_3 A_{03} Q_3 = 0.032065 \text{ } \mu\text{g/min} = 46.2 \text{ } \mu\text{g/day}.$$

All the above results on subject J.S., as well as those on subject R.D., are summarized in Table I.

In discussing the variability of pool sizes with various iodide models (93), we mentioned the fact that it is customary to evaluate the total "volume of distribution" of a metabolite from the extrapolation of the final or "equilibrated" portion of the corresponding plasma disappearance curve. Comparative pool sizes for models I, II, III, and for curve extrapolation, for iodide metabolism have been given in Table IV of paper II (93). Similar values for extrathyroidal thyroxine system are given for two subjects in Table II. Here, since  $k_1, k_3 \gg k_2$ , the extrapolation equations become:

$$Q_1 + Q_2 = \frac{Q_1}{b_1 + c_1} \quad \text{and} \quad Q_1 + Q_2 + Q_3 = \frac{Q_1}{c_1},$$

and therefore,

$$Q_2 = \frac{b_1}{c_1(b_1 + c_1)} Q_1 \quad \text{and} \quad Q_3 = \frac{a_1}{b_1 + c_1} Q_1.$$

As was previously mentioned (93), the extrapolation method for evaluation of pool sizes is not mathematically valid. (Note that the representation of the

pools in terms of the micrograms of the metabolite, rather than in terms of their "volumes" of distribution, is independent of the possible variations in the concentration of the metabolite within different body compartments.) An heuristic proof of the above follows from the fact that a single approximating curve may reflect the behavior of a series of distinct pool models with correspondingly varying pool sizes (Table II and reference 93, Table IV). In practice, the extrapolated values for the total metabolite ( $Q_1 + Q_2 + Q_3$ ) contained in the three-pool systems of iodide and thyroxine metabolisms, exceed the amounts calculated on the basis of corresponding pool models by approximately ten per cent. Such devia-

TABLE II

*Comparison of Various Pool Sizes on Two Euthyroid Subjects, Based on Calculations for Models A, B, C, D and E of Extrathyroidal Thyroxine Metabolism and on Extrapolations\* from the Approximating Curves to the Plasma  $I^{131}$  Thyroxine Disappearance Data*

Subject	Method of Calculation	$Q_1$ Plasma Thyroxine Iodine Pool in $\mu\text{g}$	$Q_2$ Intrapatent Thyroxine Iodine Pool in $\mu\text{g}$	$Q_3$ Lymph Thyroxine Iodine Pool in $\mu\text{g}$	$Q_1 + Q_2 + Q_3$ in $\mu\text{K}$
J.S.	Model A	134.3	190.2	96.9	421.4
	" B	"	182.5	104.6	421.4
	" C	"	186.2	104.6	425.1
	" D	"	178.0	112.8	425.1
	" E	"	185.0	104.0	423.3
	Extrapolation from curve	"	235.2	103.4	472.9
R.D.	Model A	235.2	224.0	107.1	566.3
	" B	"	211.8	119.3	566.3
	" C	"	217.1	119.3	571.6
	" D	"	204.0	132.4	571.6
	" E	"	214.4	119.4	569.0
	Extrapolation from curve	"	262.1	114.2	611.5

\* Note that extrapolated values are not valid mathematically.

tions, however, are much greater for single pools  $Q_2$  and  $Q_3$ , and may vary even more for models with other modes of exit and entry. In any event, the failure of the extrapolation method to supply accurate pool sizes cannot be attributed to slow mixing as suggested by Ingbar and Freinkel (39).

As was mentioned before, the amount of thyroxine degraded and excreted per day is completely independent of the models considered in this paper. In addition, the respective sizes of either  $Q_2$  or  $Q_3$  do not change significantly when calculated for any one of the above models (Table I). This leads to a speculation on the dependency of these physiological parameters upon the exact dynamic interconnections or pathways between various extrathyroidal thyroxine compartments. Similar results were obtained in the studies of iron (marrow iron transit pool) (94) and iodide (93) metabolisms. That the pool  $Q_3$  as used in the preceding analysis can be actually identified with the lymph follows from com-

parison of theoretical (calculated) activity (Fig. 7) with that of lymph following an intravenous injection of  $I^{131}$  thyroxine after cannulation of the thoracic duct in the neck according to the method of Linder and Blomstrand (50) (Fig. 8). Figure 9 represents simultaneous experimental data of tracer distribution in plasma, bile and lymph after an intravenous injection of labelled sodium-l-thyroxine in a dog (16, 50). The difference in relative positions of various curves must be attributed to the fact that the calculated values in Figure 7 refer to the total fractional activities, whereas in Figures 8 and 9 they express relative con-

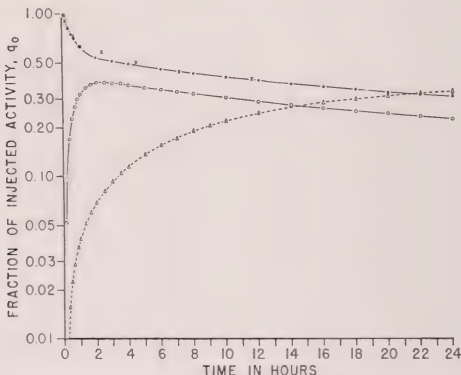


FIG. 7. Theoretical tracer distribution curves for various extrathyroidal thyroxine pools of Model A, based on the experimental  $I^{131}$  thyroxine disappearance data on subject J.S., following an intravenous injection of a tracer dose of  $I^{131}$ -labelled sodium-l-thyroxine.

- $(q_1/q_0)$  = normalized  $I^{131}$  thyroxine disappearance curve in pool  $Q_1$  (calculated).
- $(q_2/q_0)(A)$  = simultaneous tracer distribution curve of intrahepatic iodine pool  $Q_2(A)$ .
- △·△·△·△  $(q_2/q_0)(A)$  = simultaneous tracer distribution curve of the lymph thyroxine iodine pool  $Q_2(A)$ .
- ××× Normalized experimental plasma  $I^{131}$  thyroxine disappearance data.

centrations in plasma, lymph, and bile. Figure 10 represents radioactivity in plasma and in bile of the subject H.B., with acute gangrenous cholecystitis with cholecystostomy drainage tube, after an intravenous injection of  $I^{131}$  labelled sodium-l-thyroxine. Note that in this case, the activity per unit volume in the bile does not exceed significantly that in plasma. That the pool  $Q_2$  actually represents the activity in the liver seems indicated by *in vivo* counting. In Figure 11 the activity over the liver area after intravenous injection of sodium-l-thyroxine increases for several hours, while similar readings on the left side of the same normal subject do not indicate any significant increase in activity. In Figure 12, however, similar readings over the same two regions of a cirrhotic patient show no such difference.

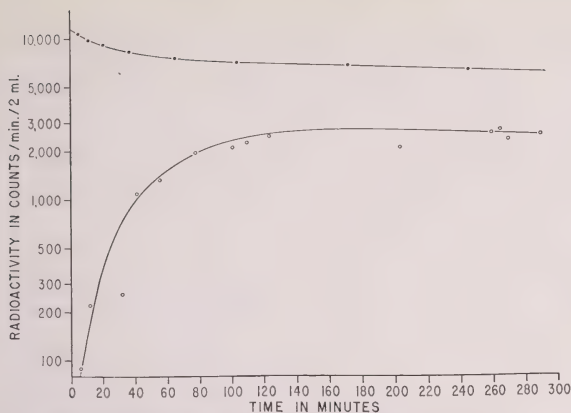


FIG. 8. Radioactivity in plasma and lymph of subject E.T., after an intravenous injection of  $I^{131}$ -labelled sodium-l-thyroxine, following the thoracic duct cannulation.

●—●—● Radioactivity in plasma.  
○—○—○ Radioactivity in lymph.

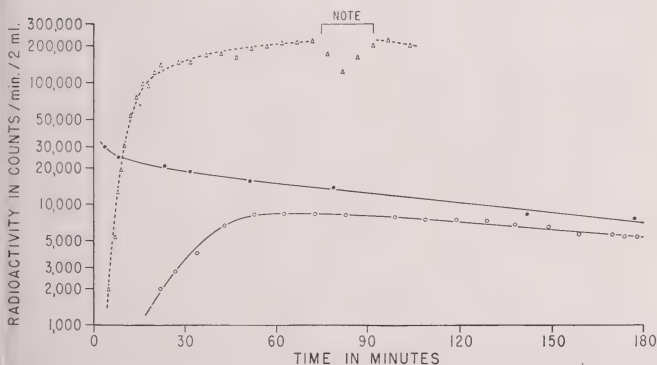


FIG. 9. Radioactivity in plasma, lymph and bile of a dog (#2077) after an intravenous injection of  $I^{131}$ -labelled sodium-l-thyroxine, following the cannulation of the thoracic duct and the transduodenal cannulation of the common bile duct.

●—●—● Activity in plasma.  
○—○—○ Activity in lymph.  
△—△—△ Activity in bile.

Note: The bile samples corresponding to 77, 82, 87, and 92 minutes, contained visible blood.

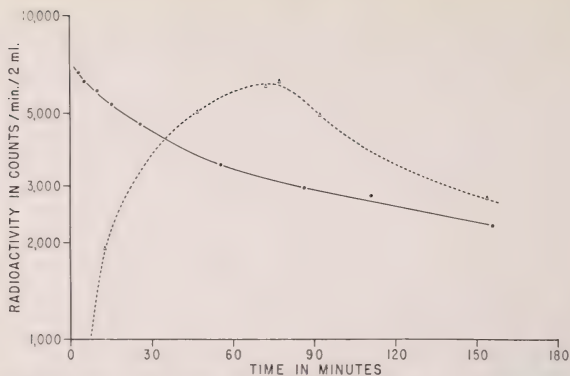
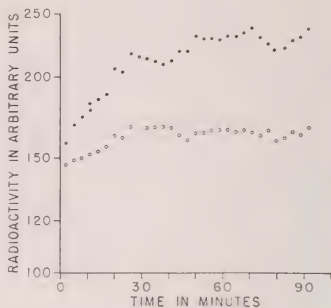


FIG. 10. Radioactivity in plasma and bile of the subject H.B. with acute gangrenous cholecystitis with cholecystostomy drainage tube, after an intravenous injection of  $I^{131}$ -labelled sodium-1-thyroxine.

●—●—● Radioactivity in plasma.  
 △---△---△ Radioactivity in bile.

FIG. 11. In vivo activity over the hepatic and splenic areas of a normal subject, after an intravenous injection of  $I^{131}$ -labelled sodium-1-thyroxine.

●●● In vivo activity over the hepatic area.  
 ○○○ In vivo activity over the splenic area.



The apparent inadequacy of limiting each study of iodine metabolism to either its inorganic or organic component, calls for more extensive studies by means of double isotopes. The method for simultaneous use of  $I^{131}$  and  $I^{124}$  has been developed in our laboratory (17) and a set of such simultaneous determinations of the activities due to  $I^{131}$  tagged thyroxine and  $I^{124}$  as iodide, both in the plasma and lymph of a dog, is demonstrated in Figure 13. Besides the usual advantages of a double isotope technique, the simultaneous study of iodide and thyroxine subsystems of iodine metabolism has specific advantages which can be subdivided into the following three categories.

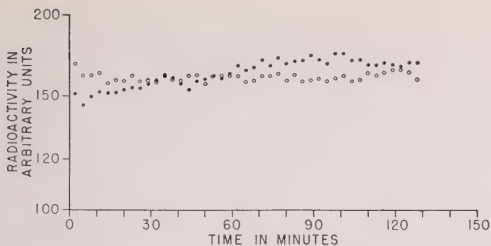


FIG. 12. In vivo activity over the hepatic and splenic areas of a subject with cirrhosis of liver, after the intravenous injection of  $I^{131}$ -labelled sodium-1-thyroxine.

●●● In vivo activity over the hepatic area.  
○○○ In vivo activity over the splenic area.

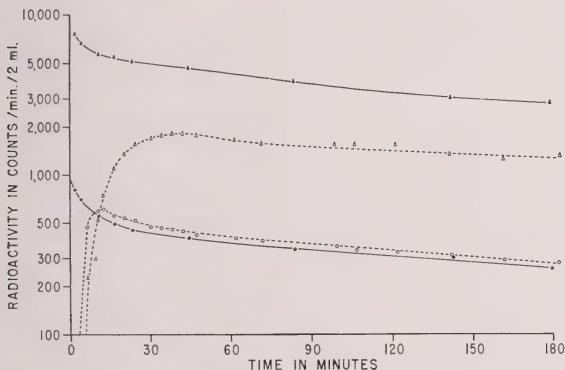


FIG. 13. Simultaneously observed  $I^{124}$  and  $I^{131}$  tracer distribution in plasma and in lymph, after an intravenous injection of sodium iodide,  $I^{124}$ , and  $I^{131}$ -labelled sodium-1-thyroxine, following cannulation of the thoracic duct in a dog:

●—●—●  $I^{124}$  activity in plasma.  
○- -○- -○  $I^{124}$  activity in lymph.  
▲—▲—▲ Thyroxine  $I^{131}$  activity in plasma.  
△- -△- -△ Thyroxine  $I^{131}$  activity in lymph.

*1. Iodide Subsystem:* In studying iodide metabolism of a given subject, we have been assuming "average values" of  $50 \mu\text{g}$  per day for hormonal degradation and  $7.5 \mu\text{g}$  per day for hormonal fecal excretion (93). A change in these values would result in a corresponding alteration of the size of the "average initial miscible iodide pool" as well as of the remaining pool constants. A simultaneous determination of plasma  $\text{PBI}^{127}$  content, as well as the determination of the true rate of hormone degradation and fecal hormone excretion of the subject by



means of a sodium-l-thyroxine tracer tagged with a second radioisotope of iodine, would automatically obviate such procedural errors.

2. *Thyroxine Subsystem*: A disappearance curve of exogenous  $I^{131}$  sodium-l-thyroxine from plasma is subject to at least one of the following errors. The thyroxine radioactivity is due not only to the initially injected thyroxine still in the circulation, but also to partial recycling of the radioiodide released from the degraded hormone. When the thyroid gland is blocked by  $I^{127}$  to prevent significant recycling of radioiodide, the system may no longer be in the original steady state, and the thyroxine degradation rate may be altered. Since the hormone degradation is probably a second order reaction (5, 79), this would change the rate of degradation of the exogenously introduced  $I^{131}$  thyroxine, giving false values for hormonal turnover rates. An independent simultaneous study of iodide metabolism by means of a second radioisotope ( $I^{124}$ ) would obviate the necessity of blocking the gland by providing a correction for the activity of the radioactive hormone due to that partial recycling of the released iodide. From the amounts of the first radioisotope (originating from exogenous hormone) released into the urine and from the study of the iodide subsystem, the daily or hourly amounts of radioactivity incorporated into the gland can be determined. The radioactive contributions of such newly formed hormone can be calculated for any given time (after the above-mentioned period of hormone degradation) by comparison with the activity of the thyroxine formed by biosynthesis and present in the plasma after the same interval of time following the injection of the second isotope (as sodium  $I^{124}$ ) into the iodide subsystem. The summation (or integration) of such corrections for all endogenous hormone formed after the initial injection of the endogenous thyroxine and bearing the same isotopic label, will give the correction necessary to account for recycling, without changing the steady-state of the system.

3. *Organic Iodine in the Thyroid Gland*: If we arbitrarily assume that all organic iodine in the thyroid gland forms a chain of *non-reversible* reactions ending in the formation and subsequent release of thyroxine, then we can approximate such a system by an infinite sequence of vanishingly small unidirectionally connected pools of similar orders of magnitude (96). The total iodine content of the gland can then be calculated from the time lag of the thyroxine appearance in plasma (i.e., the difference in time between its actual appearance in plasma and its theoretical appearance calculated on the basis of the data obtained from the solutions of the iodide and thyroxine subsystems and omitting the intervening pool comprising the gland), and the rate of thyroxine formation or equivalently, combined rate of thyroxine degradation and fecal excretion from the extrathyroidal space. A double isotope study would furnish the necessary data. In the above concept of a thyroid gland, the hormone is treated as the final step of a series of reactions immediately preceding its release into the circulation. If, however, it forms a separate compartment, i.e., a distinct pool, additional assumptions must be made before such system can be treated or possibly solved mathematically. The uncertainty as to exact biological processes within the thyroid gland, or possible release into the circulation and behavior of the iodi-

nated organic compounds is best expressed in the words of Ingbar and Freinkel (40): "We are well aware that if you give radioactive iodine to a human . . . only a relatively small fraction of this radioactive iodine appears as thyroxine within the thyroid gland. Most of it appears among the iodinated tyrosines. What happens when this hormone is released to the circulation? Presumably, the iodinated tyrosines undergo deiodination and the iodide liberated thereby is either released to the exterior or reutilized in the thyroid, thus the gland may be taking up some 200  $\mu$ g of iodine a day, but may not be putting all of that into thyroxine, since much of the iodine which enters the gland is present as iodo-tyrosines at the time that hydrolysis and deiodination occur. The same factors unquestionably apply in release experiments. Here, we are measuring two processes. First, we are unquestionably observing the release of labelled thyroxine and triiodothyronine. However, as proteolysis occurs,  $I^{131}$ -labelled iodo-tyrosines will be deiodinated and some of the loss of radioactivity from the gland which we observe clinically may be due to the loss of  $I^{131}$ -iodide arising in this manner. There is considerable and increasing evidence that not all the iodine produced by intraglandular deiodination is retained in the gland. Therefore, we would not expect total iodide accumulation or disappearance to coincide numerically with the turnover or release of thyroxine."

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## Studies in Iodine Metabolism

### IV. Formulation and Analysis of Three-Pool Systems\*

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In order to formulate mathematical models or analogues of a metabolic system, it is necessary to define fundamental assumptions about the distribution and movement of the given metabolite within the body. When tracer substances are used as the principal tool of investigation, these assumptions or postulates must not only be consistent with the known physiological properties of the metabolite, but must also permit predictions about the dynamic behavior of the tracer.

In practice only a limited number of the compartments or pools of a metabolic system are accessible to "instantaneous" introduction of a tracer substance, and to its subsequent sampling and measurement. In "Studies in Iron Kinetics III" (3) we discuss in detail how the tracer behavior in certain metabolic systems (in particular the plasma  $\text{Fe}^{59}$  disappearance after an "instantaneous" intravenous injection of  $\text{Fe}^{59}$ -transferrin), can be best† approximated by sums of exponential functions with constant coefficients. These functions form solutions of linear homogeneous differential equations with constant coefficients. Therefore, the tracer behavior within a workable mathematical model of a metabolic system can be described by a set of such differential equations.‡ These equations are solved in the standard manner by means of the Laplace transforms§ (2, 3, 11).

The general methodology underlying the formulation of multiple-pool models, and their mathematical treatment are discussed in greater detail in other papers (3, 7, 8, 9, 10, 11). In the present context we are interested primarily in formulating and handling three-pool models which are directly applicable to either iodide (5) or thyroxine (6) kinetics.

#### PART I

We will primarily consider models of metabolic systems (or subsystems||) consisting of three distinct interchanging pools ( $Q_1$ ,  $Q_2$  and  $Q_3$ ), i.e., three dis-

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† i.e., in terms of a smallest number of simple functions.

‡ The number of differential equations should not exceed the number of the distinct component pools of the system (10).

§ The mathematical treatment presented later in the text can be found in any of the appropriate standard textbooks. It is hoped, however, that the following simplified exposition may enable the interested reader to apply this mathematical analysis to his own experimental data.

The system describing iodine metabolism can be separated into three distinct parts: Iodide subsystem, thyroxine in the thyroid gland and extra-thyroidal thyroxine subsystem.

tinet aggregates of the metabolite which differ from each other either by virtue of anatomical location or physico-chemical state in which the substance occurs. Such a representation is too general, however, and in order to describe the dynamics of a metabolic model in simple mathematical terms, it becomes necessary to postulate a series of assumptions or limiting conditions.

It is assumed that at least one of the three pools is "accessible," which means that a tracer,  $q_0$ , of the metabolite can be introduced instantaneously into this pool at zero time, and representative samples can be subsequently withdrawn at desired intervals of time.

It is further assumed that the model is a reduced three-pool system (10) i.e., some of the metabolite from any one of the pools is transferred, though not necessarily directly, into the other two. All mixing is assumed to be instantaneous (10), so that for a sufficiently small interval of time the same fraction of the tracer as of the stable isotope leaves a given pool, or is transferred from one pool to another. This implies that the behavior of the tracer in each of the three pools, which is described by the tracer distribution functions  $q_1(t)$ ,  $q_2(t)$ , and  $q_3(t)$ , reflects the dynamics of the stable metabolite as a whole.

It is assumed that the metabolic system represents either first order reactions only or is in a dynamic equilibrium. A first order reaction implies that the rate with which the substance is transferred from one pool to another (or to the outside), or the rate of reaction changing its chemical properties, is directly proportional to the size of the pool from which the substance originates. The reaction is of second order if its rate is proportional to the square of the amount of the metabolite present in the corresponding pool, etc. If a system is in dynamic equilibrium and, therefore, equal amounts of the substance leave and enter each of the pools respectively, the order of the underlying reactions no longer matters, since all transfer rates of the stable metabolite, and hence those of the tracer substance, are directly proportional to the amounts present in the respective pools. The tracer distribution within such a system can be described in terms of three homogenous linear differential equations with constant coefficients.

The following notation and definitions are used (see Figures 1 and 2).

$Q_1$ ,  $Q_2$  and  $Q_3$ ;  $Q_i$  and  $Q_i(t)$  denote both the  $i^{\text{th}}$  pool and the amounts of the metabolite in the pool  $Q_i$  at the time  $t$ . When the system is in dynamic equilibrium  $Q_i(t) = Q_i = \text{constant}$ .

$k_1$ ,  $k_2$  and  $k_3$ ;  $k_i$  is the relative rate with which the substance leaves the pool  $Q_i$ . Thus, during the dynamic equilibrium,  $k_1 Q_1$  for instance is the amount of the metabolite leaving  $Q_1$  per unit time and equals a constant.

$A_{ij}$ ; A constant fraction,  $A_{ij}$ , of the amount of the substance leaving the pool  $Q_i$  is transferred to another pool  $Q_j$  with the absolute instantaneous rate  $A_{ij} k_i Q_i(t)$ . In the steady state, for instance, the constant amount  $A_{23} k_3 Q_3$  is transferred from pool  $Q_3$  to pool  $Q_2$  per unit time.

$A_0$  and  $E$ ; A constant fraction,  $A_0$ , of the amount that leaves pool  $Q_i$  is eliminated entirely from the three-pool system at an instantaneous rate  $A_0 k_i Q_i(t) = E_i(t)$ . When the system is in the dynamic equilibrium this rate becomes a constant and, for instance, the amount  $A_0 k_2 Q_2 = E_2$  leaves the

three-pool system from pool  $Q_2$  per unit time.  $E_1 + E_2 + E_3$  is the total amount of the metabolite leaving the three-pool system per unit time. In addition, for each  $i$ ,

$$\sum_{\substack{j=0 \\ j \neq i}}^3 A_{ji} = 1.$$

When  $i = 1$ , this becomes  $A_{01} + A_{21} + A_{31} = 1$ .

FIG. 1. Schematic representation of a general reduced three-pool model.

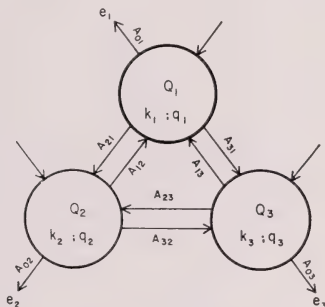
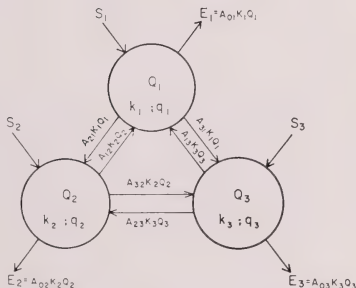


FIG. 2. Schematic representation of a general reduced three-pool system.

Here the actual amounts of the metabolite transferred from one pool to another, or leaving the system as a whole, are indicated by annotated arrows.



$S_1$ ,  $S_2$  and  $S_3$ ;  $S_i$  is the amount of the metabolite entering pool  $Q_i$  of a steady state system per unit time:  $S_1 + S_2 + S_3 = E_1 + E_2 + E_3$ .

$q_i(t)$ ,  $q_2(t)$  and  $q_3(t)$ ;  $q_i(t)$  stands for the amount of tracer contained in pool  $Q_i$  at the time  $t$ . If the tracer is radioactive, each  $q_i(t)$  represents the amount of the isotope corrected for decay with reference to a single standard time.

$q_i(0)$  and  $q_0$ ;  $q_i(0)$  is the amount of tracer which is introduced instantaneously into the pool  $Q_i$  at the time  $t = 0$ . In the following we will consider only the case where the tracer isotope is introduced into a single pool. Let  $Q_1$  be such an

accessible pool, then  $q_1(0) = q_0$  and  $q_2(0) = q_3(0) = 0$ . In the following  $q_i$  is always understood to mean  $q_i(t)$ .

$c_1$ ,  $c_2$  and  $c_3$ ;  $c_i$  is the fraction of  $q_0$  which is eventually eliminated from the system with  $E_i$ .

$\lambda_1$ ,  $\lambda_2$ ,  $\lambda_3$ ,  $a_i$ ,  $b_i$  and  $c_i$ ; It will be shown that the solution of each  $[q_i(t)]$   $q_0$  is a sum of three exponential functions with constant coefficients. The three exponents are  $-\lambda_1 t$ ,  $-\lambda_2 t$  and  $-\lambda_3 t$  (where  $t$  is time) and the corresponding coefficients are  $a_i$ ,  $b_i$  and  $c_i$ , such that for  $i = 1$ ,  $a_1 + b_1 + c_1 = 1$  and for  $i = 2$  or 3

$$a_2 + b_2 + c_2 = a_3 + b_3 + c_3 = 0.$$

$$\frac{q_1(t)}{q_0} = a_1 e^{-\lambda_1 t} + b_1 e^{-\lambda_2 t} + c_1 e^{-\lambda_3 t}.$$

In the above, all relative transfer rates,  $k_i$ , and the interchange constants,  $A_{ji}$ , apply both to the tracer and the non-tracer material.

The three differential equations describing the tracer behavior of three-pool systems subject to all the above assumptions and definitions, become

$$\frac{dq_1}{dt} = -k_1 q_1 + A_{12} k_2 q_2 + A_{13} k_3 q_3$$

$$\frac{dq_2}{dt} = A_{21} k_1 q_1 - k_2 q_2 + A_{23} k_3 q_3$$

$$\frac{dq_3}{dt} = A_{31} k_1 q_1 + A_{32} k_2 q_2 - k_3 q_3.$$

The simplest way to solve these equations is by means of Laplace transforms. (1, 2, 4, 12) In the following we will use this method (which can be applied to equations describing any number of pools) in the form of a prescription, introducing as categorical statements without proof all needed definitions and theorems. Whenever possible, such definitions and theorems will be quoted directly from Jaeger's monograph on Laplace Transformation (4).

*Definition of a Laplace transform (Jaeger, p. 1):*

"We suppose  $y(t)$  to be a known function of  $t$  for values of  $t > 0$ . Then Laplace transform  $\bar{y}(p)$  of  $y(t)$  is defined as

$$\bar{y}(p) = \int_0^\infty e^{-pt} y(t) dt,$$

where  $p$  is a number sufficiently large to make the integral convergent." A table of Laplace transforms of various functions,  $y(t)$ , and hence of functions corresponding to various transforms, can be set up by using the above definition and integrating various functions. For instance:

TABLE I

$y(t)$	$\bar{y}(p)$
1	$\frac{1}{p}$
$e^{at}$	$\frac{1}{p-a}$
$e^{-at}$	$\frac{1}{p+a}$
etc.	etc.

*Theorem (Jaeger, p. 14, theorem III):* "If  $\bar{y}(p)$  is the Laplace transform of  $y(t)$  and  $y(t)$  is continuous and tends to  $y_0$  as  $t \rightarrow 0$ , then the Laplace transform of  $dy/dt$  is  $p\bar{y}(p) - y_0$ ." This theorem (together with the fact that since Laplace transforms are integrals, the Laplace transforms of sums can be written as sums of Laplace transforms) can be applied directly to the differential equations describing the three-pool systems (or any  $n$ -pool system): each term of the equations can be replaced by its Laplace transform and each derivative  $[dq_i(t)]/dt$  by  $p\bar{q}_i(p) - q_i(0)$ . Since  $q_1(0) = q_0$  and  $q_2(0) = q_3(0) = 0$ , this becomes

$$p\bar{q}_1(p) - q_0 = -k_1\bar{q}_1(p) + A_{12}k_2\bar{q}_2(p) + A_{13}k_3\bar{q}_3(p)$$

$$p\bar{q}_2(p) = A_{21}k_1\bar{q}_1(p) - k_2\bar{q}_2(p) + A_{23}k_3\bar{q}_3(p)$$

$$p\bar{q}_3(p) = A_{31}\bar{q}_1(p) + A_{32}k_2\bar{q}_2(p) - k_3\bar{q}_3(p)$$

or

$$(p + k_1)\bar{q}_1(p) - A_{12}k_2\bar{q}_2(p) - A_{13}k_3\bar{q}_3(p) = q_0$$

$$-A_{21}\bar{q}_1(p) + (p + k_2)\bar{q}_2(p) - A_{23}k_3\bar{q}_3(p) = 0$$

$$-A_{31}\bar{q}_1(p) - A_{32}k_2\bar{q}_2(p) + (p + k_3)\bar{q}_3(p) = 0$$

The above is a set of three first degree equations in three unknowns and can be solved for any one of the variables (for instance  $\bar{q}_1(p)$ ) by means of determinants (1),

$$\bar{q}_1(p) = \frac{\begin{vmatrix} q_0 & -A_{12}k_2 & -A_{13}k_3 \\ 0 & p + k_2 & -A_{23}k_3 \\ 0 & -A_{32}k_2 & p + k_3 \end{vmatrix}}{\begin{vmatrix} p + k_1 & -A_{12}k_2 & -A_{13}k_3 \\ -A_{21}k_1 & p + k_2 & -A_{23}k_3 \\ -A_{31}k_1 & -A_{32}k_2 & p + k_3 \end{vmatrix}} = \frac{N_1(p)}{D(p)}, \text{ etc.,}$$

where the numerator  $N_i(p)$  of  $\bar{q}_i(p)$  is obtained by replacing the  $i^{\text{th}}$  column of

the determinant  $D(p)$  by  $\begin{pmatrix} q^n \\ 0 \\ 0 \end{pmatrix}$  respectively. The numerical value of a three by three determinant can be obtained by the following scheme:

$$\begin{vmatrix} a_{11} & a_{12} & a_{13} \\ a_{21} & a_{22} & a_{23} \\ a_{31} & a_{32} & a_{33} \end{vmatrix} = a_{11}a_{22}a_{33} + a_{12}a_{23}a_{31} + a_{13}a_{32}a_{21} - a_{11}a_{23}a_{32} - a_{22}a_{13}a_{31} - a_{33}a_{12}a_{21},$$

where each  $a_{ij}$  is the constant of the  $i^{\text{th}}$  row and  $j^{\text{th}}$  column. (In case of a second order determinant this is simply

$$\begin{vmatrix} a_{11} & a_{12} \\ a_{21} & a_{22} \end{vmatrix} = a_{11}a_{22} - a_{12}a_{21}.$$

The Laplace transform of  $\tilde{q}_r(p)$  has the form  $\tilde{y}(p) = [f(p)] [g(p)]$  where  $g(p)$  is of the third degree in  $p$  and  $f(p)$  is at most of the second degree in  $p$ . We can now solve directly for each  $q_r(t)$  by making use of the following theorem (Jaeger, p. 7, theorem II): "If  $\tilde{y}(p) = [f(p)] [g(p)]$ , where  $f(p)$  and  $g(p)$  are polynomials in  $p$ , the degree of  $f(p)$  being less than that of  $g(p)$ , and if

$$g(p) = (p - a_1)(p - a_2) \cdots (p - a_n),$$

where  $a_1, a_2, \cdots, a_n$  are constants, which may be real or complex but must all be different, then

$$\tilde{y}(p) = \sum_{r=1}^n \frac{1}{p - a_r} \left[ \frac{(p - a_r)f(p)}{g'(p)} \right]_{p=a_r}.$$

(The subscript  $p = a_r$  means that for each  $r$ ,  $a_r$  must be substituted for  $p$  in the expression in parentheses. The  $(p - a_r)$  of the numerator which is zero for  $p = a_r$  is cancelled out by the corresponding factor in  $g(p)$ . This can be avoided by replacing

$$\left[ \frac{(p - a)f_r(p)}{g'(p)} \right]_{p=a_r} \quad \text{by} \quad \left[ \frac{f(p)}{g'(p)} \right]_{p=a_r}$$

where  $g'(p)$  is the derivative with respect to  $p$  of  $g(p)$ .)

"Also the function  $y(t)$  whose Laplace transform is  $\tilde{y}(p)$  is

$$y(t) = \sum_{r=1}^n \left[ \frac{(p - a_r)f(p)}{g'(p)} \right]_{p=a_r} e^{-a_r t},$$

or

$$y(t) = \sum_{r=1}^n \left[ \frac{f(p)}{g'(p)} \right]_{p=a_r} e^{-a_r t}.$$

The latter part of the above theorem follows directly from Table I. In applying this theorem to the Laplace transforms

$$\bar{q}_i(p) = \frac{N_i(p)}{D(p)}$$

the factors of the denominator ( $g(p)$  of the theorem) can be obtained by setting  $D(p) = 0$  and determining the roots  $-\lambda_1$ ,  $-\lambda_2$  and  $-\lambda_3$  of the resulting polynomial.

$$\begin{aligned} D(p) &= \begin{vmatrix} p + k_1 & -A_{12}k_2 & -A_{13}k_3 \\ -A_{21}k_1 & p + k_2 & -A_{23}k_3 \\ -A_{31}k_1 & -A_{32}k_2 & p + k_3 \end{vmatrix} \\ &= p^3 + p^2(k_1 + k_2 + k_3) \\ &\quad + p[k_1k_2(1 - A_{12}A_{21}) + k_2k_3(1 - A_{23}A_{32}) + k_1k_3(1 - A_{13}A_{31})] \\ &\quad + k_1k_2k_3(1 - A_{12}A_{21} - A_{23}A_{32} - A_{13}A_{31} - A_{12}A_{23}A_{31} - A_{13}A_{32}A_{21}) \\ &= p^3 + s_1p^2 + s_2p + s_3 = (p + \lambda_1)(p + \lambda_2)(p + \lambda_3) = 0, \end{aligned}$$

where  $s_i$ 's are the "symmetric" functions

$$s_1 = \lambda_1 + \lambda_2 + \lambda_3 = k_1 + k_2 + k_3$$

$$s_2 = \lambda_1\lambda_2 + \lambda_2\lambda_3 + \lambda_3\lambda_1 = k_1k_2(1 - A_{12}A_{21})$$

$$+ k_2k_3(1 - A_{23}A_{32}) + k_1k_3(1 - A_{13}A_{31})$$

$$s_3 = \lambda_1\lambda_2\lambda_3 = k_1k_2k_3(1 - A_{12}A_{21} - A_{23}A_{32} - A_{13}A_{31} - A_{12}A_{23}A_{31} - A_{13}A_{32}A_{21})$$

(In the above  $-\lambda_1$ ,  $-\lambda_2$  and  $-\lambda_3$  correspond to  $a_1$ ,  $a_2$  and  $a_3$  of the theorem, and  $s_1$ ,  $s_2$  and  $s_3$  can be obtained in terms of  $\lambda_i$ 's by expanding the polynomial:

$$\begin{aligned} D(p) &= (p + \lambda_1)(p + \lambda_2)(p + \lambda_3) \\ &= p^3 + (\lambda_1 + \lambda_2 + \lambda_3)p^2 + (\lambda_1\lambda_2 + \lambda_2\lambda_3 + \lambda_3\lambda_1)p + \lambda_1\lambda_2\lambda_3 \end{aligned}$$

The derivatives of  $D(p)$  at  $p = -\lambda_i$  become:

$$[D'(p)]_{p=-\lambda_1} = (\lambda_1 - \lambda_2)(\lambda_1 - \lambda_3)$$

$$[D'(p)]_{p=-\lambda_2} = -(\lambda_1 - \lambda_2)(\lambda_2 - \lambda_3)$$

and

$$[D'(p)]_{p=-\lambda_3} = (\lambda_1 - \lambda_3)(\lambda_2 - \lambda_3)$$

respectively.

It will be assumed that  $\lambda_1 > \lambda_2 > \lambda_3$ . If the initial tracer was introduced into pool  $Q_1$ ,  $N_1(p)$  becomes:

$$\begin{aligned} N_1(p) &= \begin{vmatrix} q_0 & -A_{12}k_2 & -A_{13}k_3 \\ 0 & p + k_2 & -A_{23}k_3 \\ 0 & -A_{32}k_2 & p + k_3 \end{vmatrix} = q_0 \begin{vmatrix} p + k_2 & -A_{23}k_3 \\ -A_{32}k_2 & p + k_3 \end{vmatrix} \\ &= q_0[p^2 + p(k_2 + k_3) + k_2k_3(1 - A_{23}A_{32})]. \end{aligned}$$



The normalized solution of  $q_1(t)$  then becomes:

$$\begin{aligned} \frac{q_1(t)}{q_0} &= \frac{\lambda_1^2 - \lambda_1(k_2 + k_3) + k_2k_3(1 - A_{23}A_{32})}{(\lambda_1 - \lambda_2)(\lambda_1 - \lambda_3)} e^{-\lambda_1 t} \\ &\quad - \frac{\lambda_2^2 - \lambda_2(k_2 + k_3) + k_2k_3(1 - A_{23}A_{32})}{(\lambda_1 - \lambda_2)(\lambda_2 - \lambda_3)} e^{-\lambda_2 t} \\ &\quad + \frac{\lambda_3^2 - \lambda_3(k_2 + k_3) + k_2k_3(1 - A_{23}A_{32})}{(\lambda_1 - \lambda_3)(\lambda_2 - \lambda_3)} e^{-\lambda_3 t} \\ &= a_1 e^{-\lambda_1 t} + b_1 e^{-\lambda_2 t} + c_1 e^{-\lambda_3 t}. \end{aligned}$$

The other two solutions are obtained in similar manner.

In practice, some of the interchange constants,  $A_{ij}$ , may be equal to zero or to one,\* thus simplifying the manipulations of the corresponding determinants. When more than three pools are involved, the general solutions in terms of  $M_i(p)$ ,  $D'(p)$  and  $e^{-\lambda_i t}$ s can still be obtained in the same way as above, except that special care must be taken in expanding the resulting determinants (1) of orders higher than three.

## PART II

In part I we determined the equations of the tracer distribution from the known values of pool constants. In practice, the problem is just the reverse. We start with the disappearance data of a tracer substance from the accessible pool  $Q_1$ , which in our case can be approximated by a sum of three exponential functions:

$$q_1(t) = \alpha_1 e^{-\lambda_1 t} + \alpha_2 e^{-\lambda_2 t} + \alpha_3 e^{-\lambda_3 t}$$

or

$$\frac{q_1(t)}{q_0} = a_1 e^{-\lambda_1 t} + b_1 e^{-\lambda_2 t} + c_1 e^{-\lambda_3 t}$$

where  $a_1 + b_1 + c_1 = 1$  and the subscript 1 of  $a$ ,  $b$  and  $c$  refers to pool  $Q_1$ . A detailed description of the "manual" method used to obtain such approximations can be found in the "Multiple-pool analysis in tracer studies of metabolic kinetics" (10) and in the "Studies of iron kinetics IV" (7). The normalized (10) approximating equation  $[q_1(t)]/q_0$  gives the numerical values of  $\lambda_1$ ,  $\lambda_2$ ,  $\lambda_3$ ,  $a_1$ ,  $b_1$ , and  $c_1$ . Only five of these constants are independent since  $a_1 + b_1 + c_1 = 1$ . (Note that the constants  $a_1$ ,  $b_1$  and  $c_1$  are defined in terms of  $\lambda$ 's,  $k$ 's and  $A_{23}A_{32}$  in the last equation of Part I. In addition, in the case of the models A, B, C, D and E† of the extrathyroidal thyroxine metabolism (6)  $c_1$  is assumed to be equal to zero and  $c_2$  is calculated from fecal excretion data. Since  $c_2$  is the fraction

\* For instance in Model I of iodide metabolism (5) it is assumed that  $A_{22} = A_{32} = 0$ . Since, in addition, no metabolite leaves the system from either pool  $Q_2$  or  $Q_3$ , it follows that  $A_{12} = A_{13} = 1$ .

† In the following, the Models I, II, III, IV and A, B, C, D, E refer to the three-pool models of iodide (5) and extra-thyroidal thyroxine (6) metabolism, respectively.

of  $q_0$  which (with the blocked gland) is eventually excreted with  $E_2$ , it can be expressed as the integral (10)

$$e_2 = k_2 A_{02} \int_0^\infty \frac{q_2(t)}{q_0} dt.$$

Similarly,

$$e_3 = 1 - e_2 = k_3 A_{03} \int_0^\infty \frac{q_3(t)}{q_0} dt.$$

Only two of the  $e_i$ 's are independent (since  $e_1 + e_2 + e_3 = 1$ ), thus bringing the total number of known independent functions of the reduced three-pool tracer distribution system, i.e., first order tracer reaction system not involving dynamic equilibrium conditions of the stable metabolite (10), up to seven. The same is true of the models I, II, III and IV of the iodide metabolism, where it is assumed that  $e_1 = 1$  and  $e_2 = e_3 = 0$ . From Table II of the "Multiple pool analysis, I" (10), it follows that two more conditions or constraints must be imposed upon the three-pool systems in order to determine all of its relative rates and interchange constants, i.e.  $k_1$ ,  $k_2$ ,  $k_3$ ,  $A_{21}$ ,  $A_{31}$ ,  $A_{12}$ ,  $A_{32}$ ,  $A_{13}$  and  $A_{23}$  (the value of each  $A_{0i}$  follows from the defining equations:

$$A_{0i} = 1 - \sum_{\substack{j=1 \\ j \neq i}}^{j=3} A_{ji}).$$

In models I, II and III of the iodide subsystem and in models A, B, C and D of the extrathyroidal thyroxine subsystem, the two additional constraints are obtained by equating two of the interchange constants to zero.

For a general three-pool system with the accessible pool  $Q_1$  (i.e., both introduction of the tracer and subsequent sampling refer to  $Q_1$ ) and a given value of  $A_{23}A_{32}$  (note that  $A_{23}A_{32} = 0$  for models I, II, III, A, B and C), the pool constants  $k_1$ ,  $k_2$  and  $k_3$  can be obtained as follows from the definitions of  $a_1$ ,  $b_1$  and  $c_1$  (see last equation of part I):

$$a_1 = \frac{\lambda_1^2 - \lambda_1(k_2 + k_3) + k_2k_3(1 - A_{23}A_{32})}{(\lambda_1 - \lambda_2)(\lambda_1 - \lambda_3)}$$

$$b_1 = \frac{\lambda_2^2 - \lambda_2(k_2 + k_3) + k_2k_3(1 - A_{23}A_{32})}{(\lambda_1 - \lambda_2)(\lambda_2 - \lambda_3)}$$

$$c_1 = 1 - a_1 - b_1.$$

Clearing fractions we get:

$$(1 - A_{23}A_{32})k_2k_3 - \lambda_1(k_2 + k_3) = a_1(\lambda_1 - \lambda_2)(\lambda_1 - \lambda_3) - \lambda_1^2$$

$$(1 - A_{23}A_{32})k_2k_3 - \lambda_2(k_2 + k_3) = -b_1(\lambda_1 - \lambda_2)(\lambda_2 - \lambda_3) - \lambda_2^2.$$

Subtracting, and dividing by  $(\lambda_2 - \lambda_1)$ :

$$\begin{aligned} k_2 + k_3 &= -a_1(\lambda_1 + \lambda_3) - b_1(\lambda_2 - \lambda_3) + \lambda_1 + \lambda_2 \\ &= a_1(\lambda_2 + \lambda_3) + b_1(\lambda_1 + \lambda_3) + (1 - a_1 - b_1)(\lambda_1 + \lambda_2) \end{aligned}$$

or

$$k_2 + k_3 = a_1(\lambda_2 + \lambda_3) + b_1(\lambda_1 + \lambda_3) + c_1(\lambda_1 + \lambda_2) = X_1.$$

Similarly, after multiplying the above cleared fractions by  $\lambda_2$  and  $\lambda_1$  respectively, we get:

$$k_2 k_3 (1 - A_{22} A_{32}) = a_1 \lambda_2 \lambda_3 + b_1 \lambda_1 \lambda_3 + c_1 \lambda_1 \lambda_2 = X_2$$

When  $A_{22} A_{32} = 0$ , as is the case for models I, II, III, A, B and C, the last equation reduces to

$$k_2 k_3 = a_1 \lambda_2 \lambda_3 + b_1 \lambda_1 \lambda_3 + c_1 \lambda_1 \lambda_2 = X_2$$

and the  $k_i$ 's can be calculated by means of the following formula:

$$k_2 = \frac{X_1 \pm \sqrt{X_1^2 - 4X_2}}{2} = r_1, r_2.$$

In case of models I and A, both values of  $k_2$  are possible, i.e., either  $k_2 = r_1$  and  $k_3 = r_2$  or  $k_2 = r_2$  and  $k_3 = r_1$ . In the remaining models, only one set of values for  $k_2$  and  $k_3$  is possible:  $k_2 > k_3$  in models II and III and  $k_3 > k_2$  in models C and D. The value of  $k_1$  follows from the definition of  $s_1$  in part I:

$$k_1 = s_1 - (k_2 + k_3) = \lambda_1 + \lambda_2 + \lambda_3 - (k_2 + k_3).$$

The solution for  $k_2$  and  $k_3$  of model D is derived elsewhere (10). The remaining constants  $A_{ij}$ 's and  $A_{0i}$ 's are determined as described in considerable detail in the "Multiple-pool analysis in tracer studies of metabolic kinetics," (10, part II) and actual solutions for various three-pool models of iodide, thyroxine and iron subsystems can be found in the corresponding papers (5, 6, 7). Here we will consider a more general case, where no interchange constants are zero, and where  $A_{23}$  and  $A_{32}$  are assigned positive values  $a_{23}$  and  $a_{32}$  respectively:  $0 < a_{23}, a_{32} < 1$  (Note that not all such values of  $a_{23}$  and  $a_{32}$  may be possible, i.e., the tracer distribution system may have solutions only for limited ranges of such values.)

When  $A_{23} = a_{23}$  and  $A_{32} = a_{32}$  the values of  $k_2$ ,  $k_3$  and  $k_1$  can be obtained as above from

$$k_2 + k_3 = a_1(\lambda_2 + \lambda_3) + b_1(\lambda_1 + \lambda_3) + c_1(\lambda_1 + \lambda_2) = X_1$$

$$k_2 k_3 = \frac{a_1 \lambda_2 \lambda_3 + b_1 \lambda_1 \lambda_3 + c_1 \lambda_1 \lambda_2}{1 - a_{23} a_{32}} = \frac{X^2}{1 - a_{23} a_{32}}$$

and

$$k_1 = s_1 - (k_2 + k_3).$$

From the expanded form of  $D(p)$  and the definitions of  $s_i$ 's given in the first part, it follows that:

$$1 + \frac{k_2(1 - A_{12} A_{21})}{k_1} + \frac{k_3(1 - A_{13} A_{31})}{k_1} = \frac{s_2 - k_2 k_3 (1 - a_{23} a_{32})}{k_1}$$

$$2) \quad (1 - a_{23}a_{32}) - A_{12}A_{21} - A_{13}A_{31} - a_{23}A_{12}A_{31} - a_{32}A_{13}A_{21} = \frac{s_3}{k_1k_2k_3} = Y.$$

Equation 2) defines  $Y$ . The expression for  $e_i$  can be obtained from the integral

$$e_i = A_{0i}k_i \int_0^\infty \frac{q_i(t)}{q_0} dt.$$

Substituting for  $q_i, q_0$  the solutions given in part I and integrating (for details

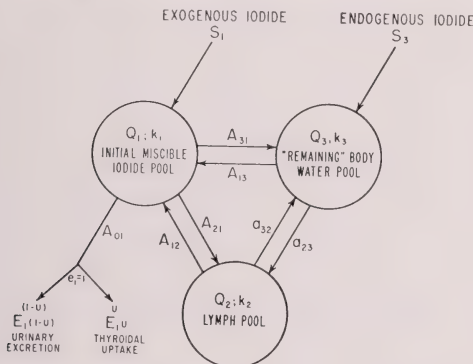


FIG. 3. Schematic representation of iodide metabolism Model IV.

see ref. 10), we get for the general case,

$$e_1 = \frac{A_{01}(1 - A_{23}A_{32})k_1k_2k_3}{s_3}$$

$$e_2 = \frac{A_{02}(A_{23}A_{31} + A_{21})k_1k_2k_3}{s_3}$$

$$e_3 = \frac{A_{03}(A_{32}A_{21} + A_{31})k_1k_2k_3}{s_3}.$$

In the case of model IV of iodide metabolism (5)  $e_1 = 1$  and  $e_2 = e_3 = A_{02} = A_{03} = 0$ . Then

$$A_{01} = \frac{e_1 s_3}{k_1k_2k_3(1 - A_{23}A_{32})} = \frac{Y}{1 - a_{23}a_{32}}.$$

$$A_{12} = 1 - a_{32}$$

$$A_{13} = 1 - a_{23}.$$

Substituting these values for  $A_{12}$  and  $A_{13}$  into the equation 1) we get

$$k_2[1 - (1 - a_{32})A_{21}] + k_3[1 - (1 - a_{23})A_{31}] = \frac{s_2 - k_2k_3(1 - a_{23}a_{32})}{k_1}$$

From this and from the identity  $A_{31} = 1 - A_{21} - A_{01}$  it follows that

$$A_{21} = \frac{k_1k_2 + k_1k_3[a_{23} + (1 - a_{23})A_{01}] - s_2 + X_2}{k_1[k_2(1 - a_{32}) - k_3(1 - a_{23})]}$$

and finally

$$A_{31} = 1 - A_{21} - A_{01}.$$

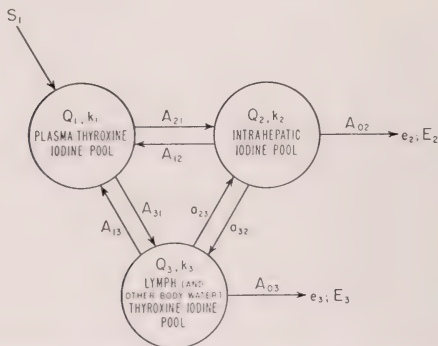


FIG. 4. Schematic reference of extra-thyroidal thyroxine metabolism Model E.

In the case of model E of extra-thyroidal thyroxine metabolism (6)

$$e_2 = \frac{A_{02}(a_{23}A_{31} + A_{21})k_1k_2k_3}{S_2}$$

or

$$3) \quad A_{02} = \frac{e_2 Y}{a_{23}A_{31} + A_{21}}.$$

Similarly

$$4) \quad A_{03} = \frac{e_3 Y}{a_{32}A_{21} + A_{31}}.$$

Also for the particular mode of exit assumed, i.e., for  $e_1 = E_1 = 0$ :

$$5) \quad A_{31} + A_{21} = 1 \quad \text{or} \quad A_{31} = 1 - A_{21}$$

$$6) \quad A_{12} + A_{32} + a_{02} = 1 \quad \text{or} \quad A_{02} = (1 - a_{02}) - A_{12}$$

$$7) \quad A_{13} + A_{03} + a_{23} = 1 \quad \text{or} \quad A_{03} = (1 - a_{23}) - A_{13}.$$

Using the last three expressions to eliminate  $A_{21}$ ,  $A_{02}$  and  $A_{03}$ , the equations 1), 3) and 4) become:

$$1a) \quad k_2(1 - A_{12}A_{21}) + k_3(1 - A_{13} + A_{13}A_{21}) = \frac{s_2 - k_2k_3(1 - a_{23}a_{32})}{k_1}$$

$$3a) \quad A_{02} = (1 - a_{32}) - A_{12} = \frac{e_2Y}{a_{23}A_{31} + A_{21}} = \frac{e_2Y}{a_{23} + (1 - a_{23})A_{21}}$$

or

$$A_{12} = \frac{a_{23}(1 - a_{32}) + (1 - a_{32})(1 - a_{23})A_{21} - e_2Y}{a_{23} + (1 - a_{23})A_{21}}$$

$$4a) \quad A_{03} = (1 - a_{23}) - A_{13} = \frac{e_3Y}{a_{32}A_{21} + 1 - A_{21}}$$

or

$$A_{13} = \frac{(1 - a_{23}) - (1 - a_{23})(1 - a_{32})A_{21} - e_3Y}{1 - (1 - a_{32})A_{21}}$$

Substituting the above expressions for  $A_{12}$  and  $A_{13}$  into 1a), we finally get

$$\begin{aligned} & \left\{ \frac{(1 - a_{23})(1 - a_{32})A_{21}^2 + [a_{23}(1 - a_{32}) - e_2Y]A_{21}}{a_{23} + (1 - a_{23})A_{21}} \right\} k_2 \\ & + \left\{ \frac{(1 - a_{23})(1 - a_{32})A_{21}^2 - [(1 - a_{23})(2 - a_{32}) - e_3Y]A_{21} + 1 - a_{23} - e_3Y}{1 - (1 - a_{32})A_{21}} \right\} k_3 \\ & = k_2 + k_3 - \frac{s_2 - X_2}{k_1} \end{aligned}$$

Substituting the numerical values for  $a_{23}$ ,  $a_{32}$ ,  $e_2$ ,  $e_3$  and  $Y = s_3/k_1k_2k_3$ , and clearing the fraction results in a third degree equation in  $A_{21}$  of the form

$$aA_{21}^3 + bA_{21}^2 + cA_{21} + d = 0.$$

This can be solved for  $A_{21}$  in any of the several accepted ways. As a rule, for a given set of values,  $a_{23}$  and  $a_{32}$ , at most one solution will give acceptable values for  $A_{21}$ , i.e.,  $0 < A_{21} < 1$ . The remainder of the constants follow directly from expressions 5), 3), 4), 6) and 7). Note that  $A_{01}$  was assumed to be zero. The actual numerical calculations for this model are given in the paper on extra-thyroidal thyroxine metabolism (6).

In order to determine the amount of metabolite present in each pool or eliminated from any part of the steady state system (i.e., to define a three-pool system in dynamic equilibrium completely) six more constraints are necessary (10). Three of these are supplied by the three equilibrium conditions:

$$k_1Q_1 = S_1 + A_{12}k_2Q_2 + A_{13}k_3Q_3$$

$$k_2Q_2 = S_2 + A_{21}k_1Q_1 + A_{23}k_3Q_3$$

$$k_3Q_3 = S_3 + A_{31}k_1Q_1 + A_{32}k_2Q_2$$

In the case of the models of iodide metabolism, the remaining three conditions are represented by actual amounts of iodide entering each pool:  $S_2$ ,  $S_3$  and  $S_1 = 0$ . In the case of the models of extrathyroidal thyroxine metabolism the three conditions are fulfilled by the known value of plasma thyroxine iodine,  $Q_1$ , and by the fact that all thyroxine enters only through pool  $Q_1$ . The fractions of the total amount entering the system by means of pools  $Q_1$ ,  $Q_2$  and  $Q_3$  are 1, 0, 0, respectively. They represent only two independent conditions, since their sum must be equal to one.

The values of  $Q_2$ ,  $Q_3$ ,  $E_1$ ,  $E_2$  and  $E_3$  of a general case where  $Q_1$  is the accessible pool and where both tracer,  $q_0$ , and the stable metabolic substance enter the three-pool system only through  $Q_1$ , are obtained as follows:

$$k_2 Q_2 = k_1 A_{21} Q_1 + k_3 A_{23} Q_3$$

$$k_3 Q_3 = k_1 A_{31} Q_1 + k_2 A_{32} Q_2$$

The solutions for  $Q_2$  and  $Q_3$  become

$$Q_2 = \frac{k_1}{k_2} Q_1 \frac{A_{21} + A_{23} A_{31}}{1 - A_{23} A_{32}}$$

$$Q_3 = \frac{k_1}{k_3} Q_1 \frac{A_{31} + A_{32} A_{21}}{1 - A_{23} A_{32}}$$

and

$$E_2 = k_2 Q_2 A_{02}$$

$$E_3 = k_3 Q_3 A_{03}.$$

Note that the third equilibrium condition has not been used directly. It is, however, implicit in the assumption that  $Q_1$  is a constant.

$E_i$ 's are the rates with respect to the same unit of time as used in determining  $k$ 's. Hence, if  $k$ 's are relative rates per minute,  $E_i$ 's must be multiplied by 1440 in order to obtain daily turnover rates. In the preceding,  $A_{01}$ ,  $A_{02}$  and  $A_{03}$  were shown to be equal to

$$A_{01} = \frac{e_1 S_3}{k_1 k_2 k_3 (1 - A_{23} A_{32})},$$

$$A_{02} = \frac{e_2 S_3}{k_1 k_2 k_3 (A_{21} + A_{23} A_{31})}$$

and

$$A_{03} = \frac{e_3 S_3}{k_1 k_2 k_3 (A_{31} + A_{32} A_{21})}$$

respectively. Hence



$$E_1 = k_1 Q_1 A_{01} = \frac{k_1 e_1 s_3 Q_3}{k_1 k_2 k_3 (1 - A_{23} A_{32})} = \frac{e_1 s_3 Q_1}{X_2}$$

$$E_2 = \frac{e_2 s_3 Q_1}{X_2}$$

$$E_3 = \frac{e_3 s_3 Q_1}{X_2}$$

and, therefore:

$$E_1 + E_2 + E_3 = \frac{s_3 Q_1}{X_2}.$$

When all stable substance enters the system through pool  $Q_2$  and/or  $Q_3$ , the equation for  $E_1$  remains the same as above, while those for  $E_2$  and  $E_3$  change. This can be summarized as follows:

Let the tracer  $q_0$  be introduced instantaneously into pool  $Q_1$ . Then, if all stable metabolite enters the three-pool system as  $S_1$ ,  $E_i$ 's are proportional to the corresponding  $e_i$ 's, and if sampling takes place from  $Q_1$ ,  $E_i = (e_i s_3 / X_2) Q_1$  for all  $i$ , and  $E_1 + E_2 + E_3 = (s_3 / X_2) Q_1$ . In addition, if  $q_0$  is introduced into the sampling pool  $Q_1$ ,  $E_1 = (e_1 s_3 / X_2) Q_1$  as before, regardless of the mode of entry of the stable isotope, though the proportionality between  $E_i$  and  $e_i$  no longer holds for  $S_2 \neq 0$  and/or  $S_3 \neq 0$ .

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## Computer Learning and the Scientific Method: A Proposed Solution to the Information Theoretical Problem of Meaning

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### ABSTRACT

This discussion outlines and implements the theory of an inductive inference technique that automatically discovers classes among large numbers of input patterns, generates operational definitions of class membership with explicit levels of confidence, creates a continuously updated "self-organized" coded hierarchical taxonomic classification of patterns, and recognizes to which already discovered class or classes, if any, a new input belongs in an information-theoretically efficient way. Relationships to the "scientific method" and learning are discussed.

Learning processes which provide the individual and his species with an increased potential to cope with his material, biological and social environment have what evolutionists call "adaptive value" (e.g., see Dobzhansky (1)). Measured on such a scale, the most important aspect of such learning processes is the development of implicit or explicit techniques to accurately estimate the probabilities of future events. And, since the "calculus" of future events from analysis of past experience is the forte of science, it seems reasonable to turn to the methods of science for some useful clues when searching for efficient learning techniques. Modern scientists, and physical scientists in particular, who work regularly with the scientific method and operationalism (2), are keenly aware of some very efficient and sophisticated techniques for learning more about the universe. But if we ask of these who, it seems, should best understand the learning process, "How shall we build—or indeed, is it possible to build—a machine that can learn and think?", the replies are varied (3-8), and, as is indicated in a recent knowledgeable review by Selfridge (9), so far have been relatively unproductive.

In this discussion, I hope to provide a basis for establishing considerably greater confidence in the broad potentials for machine learning via automated inductive processes. This will be attempted by sketching out, in operational terms and against a background of common experience, many theoretical and

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a few of the practical problems. These will include problems concerning concepts such as "gestalt," "correspondence of attributes," "similarity," "natural groupings," "classification," "operational definition," "discovery," "hypothesis," "theory," "measure of confidence," "experiment," and "causality," all of which I believe can serve useful roles in increasing understanding of learning by inductive processes. Such practical problems as those relevant to the choice of largely "parallel" as opposed to "sequential" computers for implementing such processes will also be briefly examined.

Some solutions to those problems which appear pivotal will be developed and these will be incorporated into a simple set of instructions for an adaptive pattern recognition program for a digital computer which serves as a workable model of the inductive process. This model will be evaluated with some novel information theoretical measures which permit us to maximize the "naturalness" of the "discovered" classes. Whereas the reader should have little difficulty in assimilating much of what is presented in most other sections of this paper, some difficulty with the development of this particular evaluation may be experienced by those who lack some familiarity with Information Theory.

Weaver (10) has defined three levels of Information Theory (also called the Theory of Communication): the Technical Level, the Semantic Level, and the Influential Level. Information Theory has, so far, dealt successfully with some technical problems concerning the processing of information, i.e., problems related to the question, "With what accuracy and efficiency can symbols of communication be transmitted?" and has dealt less successfully with others related to such questions as, "What practical devices can permit both the most accurate and cheapest communication of such symbols?" Until now, such semantic problems as those raised by the questions, "What are the meanings of these symbols?" and "How can the meanings be accurately and economically communicated?" as well as problems of intention raised by such questions as, "How can we most effectively insure that a message will fulfil the purpose of our communication?" have remained as they were when first examined in this context by Weaver, largely untouched. In developing answers to these latter questions, the "information" in Information Theory will begin to have a meaning much closer to the information of every day usage and interest.

Our model will be shown to provide a useful bridge between the technical and semantic levels of Information Theory because it "discovers" "meanings," it provides a useful approximation to the "ideal coder and decoder," the possibility of which is predicted by Shannon's Binary Coding Theorem (10), and it seems to begin to answer, at both the technical and semantic levels, the question of how to accurately and economically communicate symbols and meanings. (This model will also demonstrate that the problem of meaning is no longer "irrelevant to the engineering (technical) problem" (see Shannon (10) and Brillouin (11)).) Connections to the Influential Level of Information Theory will be indicated in our discussion of the concept of "experiment."

Some clues to possible solutions to most other problems discussed will also be briefly sketched in.

The widespread application of computers to the solution of human problems will remove the individual citizen further and further from crucial decision making processes. Before the solution of such problems is relegated, perhaps irrevocably, to an impersonal but "superior" mechanical intelligence (as some believe is ultimately both desirable and, in any case, inevitable), it will be valuable to be able to convey some understanding of the mechanical decision making process to the average intelligent person. This may be necessary if this new technology is to receive his full support and if those fears which stem from ignorance of the methods of the machine (and the methods of science) are to be lessened. His attention must also be brought to focus on the need for solutions to the newly created problems of "cultural adaptation" of man and machine to one another.

Because this model hews closely to ordinary intuition in many areas of its development, it may also be able to serve such a general educational need. Though this may be of some value in the short run in so far as it might catalyze interest in, and support for, needed technological and social research and development, in the long run, only proven utility of particular machine approaches to inductive processes can provide a satisfactory basis for enduring confidence and support. Hopefully, some elements of this analysis will survive the test of use.

#### LEARNING AND THE MORPHOLOGICAL SCIENCES

In addition to the elements of the classical scientific method (which will be analyzed in some detail further on), science may have developed some other useful keys to efficient learning. Where in science may we hope to find these keys?

In order to help in developing an answer to this question, let us first examine our personal experiences of learning processes. These can be conveniently resolved into two (not necessarily mutually exclusive) kinds:

- a) Learning associated with the raw sensory experiences of the universe\*;  
and
- b) Learning associated with those distillations of the experiences of other human beings which are transmitted by means of social communication.

The wisdom (or folly) of others (which can not be used until experience

\* Such learning dominates the period from birth to the beginnings of an understanding of language and other social codes (pointing, grimacing and smiling, etc.). Of course, during this period as well as later on, learning is largely dependent upon whatever minimum of heritable "distillations of the experience of the species" are already built or programmed into the nervous system at birth. Such evolutionary "wisdom" is imparted through the particular structure of the nervous system and sense organs and involves both "special purpose" "instinctive" patterns of response to external and internal stimuli as well as more "general purpose" primitive "logical processing routines." The limits as well as the adaptive value of such "wisdom" are highly dependent upon the built-in bounds to the sensitivity, dynamic range, and kind and magnitude of the "field of view" of each of the sense organs.

with a minimum of input of raw data has provided rudimentary techniques for handling complex inputs like language) is all ultimately derived from raw environmental experience, even though some of it was gathered back in pre-historic times. This might well lead us to believe that hidden away in the primitive early interactions of the newborn child and his "chaotic" environment lie clues to the fundamentals of the learning process. Unfortunately, direct access to the memories of this period of our lives seems least available to us as individuals, and this kind of interaction is only beginning to be unravelled by careful observation of learning in very young children (12-15).

But among the sciences, the classical morphological sciences have a similar relation to, for example, physics or genetics, as the infant has to the adult. The morphologist is largely concerned with ordering the raw sensory stimuli presented to him by the objects which he studies. The practitioners of the "more advanced" sciences are usually concerned with higher levels of order, i.e., the structuring (in "metalanguages") of more complex relationships of raw sensory stimuli of the present and past with one another and with such previously recognized order as physical and biological "Laws." As pointed out by the mathematician-physicist, H. Weyl, (16), "... *the formation of concepts and theories by science ... is preceded by ordering and classification.* Perhaps more stress should have been laid on this preliminary stage, that still plays a major role in biology while it has become of subordinate importance in physics. The spectacle of the immense variety of plant and animal species displayed by nature has been an early and persistent stimulus for biology to develop to great perfection the *art* of morphological and taxonomic classification. The remarkable fact that the diverse species, notwithstanding the range of variation, mostly exhibit clearly recognizable typical differences, has facilitated the task. *The typical may be elusive in terms of well-defined concepts, and yet we handle it with instinctive certitude, e.g., in recognizing persons.* Nor is it easy to describe in general terms how the process of classification, step by step and ever more convincingly, succeeds in separating essential from unessential features" (italics mine).

Whereas the morphologist may not necessarily be able to present us with the plans for a learning and thinking machine, Weyl's comment suggests that a serious effort to unravel and explicate the foundations of the taxonomic-morphologic approach may be rewarding. It is my intention to demonstrate that it is relatively "easy to describe in general terms how" a "process of classification, step by step and ever more convincingly, succeeds in separating essential from unessential features," and that therein lies the foundation of the inductive learning process.

If we ask a herpetologist how he has learned to distinguish one species of frog from another—for example the leopard frog from the pickerel frog—he can hand us a taxonomic key to the Anura of North America, in which some characteristic attributes of each taxonomic group, from Order down to sub-species are collected—all in hierarchical rank (e.g., (17)). Given a specimen of each of these species—and the key—a novice would have a fair probability of properly identifying these specimens.



Yet appearances are deceiving. The experienced herpetologist himself rarely depends completely on the particular attributes in the key to identify these frogs. In the field—at 40 feet, with only  $\frac{1}{4}$  of the animal exposed to view—he may easily identify members of these closely related species with a very high degree of confidence. Any two herpetologists are likely to perform about as well in such a circumstance, yet nowhere in herpetological literature are we likely to find a complete set of rules to which each would independently subscribe as the basis for his particular method of identification.

Two pathologists studying the same poorly prepared frozen sections of a surgical biopsy of a rare disease may each be able to arrive at an accurate

FIG. 1. Mr. X.



diagnosis. Yet, if he had to rely wholly on a word description of what the other had seen, would be extremely hesitant to make a diagnosis.

In short, morphologists have canonized some sets of descriptive attributes of the objects which they study. These are not necessarily complete descriptions, and often are not the sets of attributes which they, as experts, actually use. Furthermore, when asked, they are hard put to explicate the basis of their own personal expertise at classification.

To gain some insight into this dilemma, let us examine Weyl's example of the problem of recognizing persons:

Most readers will have seen the person in Figure 1 for the first time on reading this article. If, after setting this article down, you were handed a set of one hundred different black and white photographs of caucasian male New Yorkers—including another picture of Mr. X—most of you would probably experience very little difficulty in identifying his photograph from memory. If, on the other hand, after closing these pages, a large group of you readers were



all to cooperatively write up a description of Mr. X which was to be used by another group as the sole basis for identifying his photograph from among the hundred—the frequency of correct identifications would be much much lower. In both tasks you would start with the same mental image or “gestalt.” A new image can usually be compared directly with the “gestalt” with small error. To decode the “gestalt” and translate it into words—to then visualize a new mental image from the word picture—and finally, to compare this mental image to the photograph, involves a large loss of information as well as the generation of considerable error.

#### A TRANSLATION PROBLEM

Human beings simply do not know how to formulate adequate word pictures, i.e., how to accurately translate the mental record of an optical or acoustic image into the digital symbols we call words (one can recognize a familiar voice almost as readily as a familiar face). That this is a problem of translation seems clear: A caricaturist would be able to translate his mental image of Mr. X's face into a sketch (an analogue output) which many of you would be able to use successfully to select his picture from the set, and a good mimic would do as well in the auditory realm with still another kind of analogue output. However, the “artist's” word picture of face or voice would be much less adequate.

If a key to the learning process resides in the techniques of taxonomic morphology, it should not be too surprising that it has remained hidden from general view. For the morphologists, no less than the rest of us, have marginal verbal access to their mental images, and the taxonomies they have generated are necessarily quite primitive and seem to be more a product of “art,” as Weyl put it, than of science. Their pattern recognition techniques might therefore not have been expected to inspire widespread confidence in their potentials for the construction of a satisfactory model of the learning process.

Cell biologists have been very much concerned with this problem of defining patterns—either explicitly or implicitly, because in one way or another, all their work is related back to the two-dimensional optical images of cells which they see under various kinds of microscopes. During the last 25 years, in order to explore the clearcut early evidence of a relationship between RNA and protein synthesis, and between DNA and the hereditary message, as well as to provide a way down from the growing Tower of Babel on which our two expert pathologists find themselves when discussing their frozen section, cytologists began to try to convert their microscopic observations into the universal language of numbers. In particular, a considerable and successful effort has been expended to quantitate the information about the distribution and concentrations of the various substances of which cells are composed. Microspectrophotometric techniques were devised for measuring the amount of light absorbed as a result of the natural ultraviolet absorption—or the absorption of visible wavelengths by *in situ* colored products produced in parts of cells as the result of specific chemical test reactions (see, for example (18)). It was

largely in this particular resurgence of cell biology that I first cut my scientific teeth. It is from this perspective that I first began to appreciate how central a position pattern recognition, descriptive morphology, and taxonomy hold in relation to an understanding of the general problem of learning.

#### DISC ELECTROPHORESIS AND A NEW LEAD

A few years ago, as a by-product of our cellular studies, Dr. B. J. Davis and I developed a new, reproducible, high-resolution electrophoretic technique which we call "Disc Electrophoresis" (19, 20). This technique permits the identification of over twenty proteins—and measurement of their concentrations in a sample of as little as 1 microliter of blood serum. Figure 2 shows a photograph of such a run. Position "identifies" the protein; optical density provides a measure of its concentration.

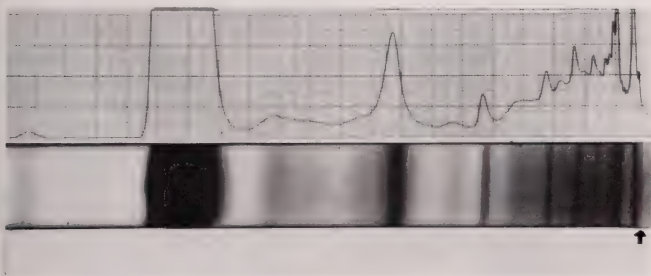


FIG. 2. Proteins of a three microliter sample of human serum, haptoglobin type 2-2. Origin indicated by arrow. Densitometric trace of the sample; abscissa, position; ordinate, optical density (2 O.D. full scale). Same run as right hand specimen in Figure 5.

Of the many potentials of this technique, the value, as a clinical diagnostic tool, of this quick quantitative check on over twenty complex substances per sample, seemed obvious, and we have been applying ourselves to the realization of this potential.

The type of analysis which the cytophotometrist performs on optical images of cells is a problem in the recognition of two-dimensional patterns. This new universe of electrophoretic patterns presents us with the one-dimensional homologue of this much more difficult two-dimensional problem. It therefore has provided us with both an opportunity and a compulsion to wade into the problem in both a serious, and, I believe, hopeful way.

In the near future, we expect to be able to collect upwards of 10,000 specimens per week—and to have the data recorded as the logarithms of the optical densities at about 400 positions per sample. These logarithms will be recorded on tape in 7 bit binary code, providing the equivalent of a constant accuracy of about 5% of concentration over a range of concentrations from about 6% (by wt.)



of a protein in the blood serum—down to about 0.006% (see Fig. 3). Without a doubt, such data will contain much useful information. The major problem was how to rapidly and efficiently process these data.

We have before us a new, unexplored, enormous but finite universe consisting of the blood sera of all living humans, each consisting of up to some 200 *resolvable* proteins in a bounded concentration range. We have a “sense organ,” the photometric apparatus, which can identify 400 positions in the pattern and measure concentration throughout the range with an essentially fixed resolution. The information about each sample is available as a sequence of 400 7 bit numbers.

How shall we learn from observing this universe?

#### THE MORPHOLOGICAL APPROACH

The morphological approach suggests that we group all the electrophoretic samples in a comprehensive class; list the attributes which all members of the class share in common; subdivide the main class into subgroups which contain members more similar to one another within each subgroup than to members of other subgroups; list the additional attributes which all members of a subgroup share in common—and continue subdividing subgroups and describing them until the subgroups cease to be easily subdivided. In this way, a taxonomic key would be generated.

Hopefully, the terminal subgroups would contain as members, the samples of sera of individuals who—on inspection, will also be found to share special physiological states in common. Many of the terminal subgroups may “define” certain diseases; some should result in the discovery or resolution of new disease entities within previously poorly defined and understood “disease groups”; and some should discover different normal genetic types. On the other hand, if the comprehensive class contains no “natural” subclasses, or if the “true” values of the data are obscured by excessive noise, for example, by error in measurement, the subgroups will not be useful.

Historically, clearly defined subgroups have generally been discovered before explicit intermediate classes have been well defined—and the taxonomic trees

FIG. 3A. Block diagram of a mechanical flying-spot scanner.  $I_0$ , background signal;  $I$ , sample signal;  $O.D.$ , optical density; 1N916, “logarithmic diodes.” The flying-spot is generated in the following manner: A very high brightness xenon arc is imaged by a microscope objective,  $M.O.$ , of numerical aperture ( $N.A.$ ) 0.3, onto the end of a clad, 50 micron diameter, 20 inch long glass fiber of  $N.A.$  0.6. This end is locked in position. The other end of the fiber is carried on the “pen” of a high-speed, rectilinear pen-galvanometer driven through a power amplifier,  $P.A.$ , by the oscilloscope sweep generator.

FIG. 3B Optical system of scanner:  $A.L.$ , achromatic lens;  $S.A.$ , slot-shaped aperture;  $M.L.$ , meniscus lens;  $B.S.$ , beam-splitter;  $P.M.$ , photomultiplier;  $P.M.C.$ , photomultiplier photocathode;  $F_1$  and  $F_2$ , filters as indicated in 3D.

FIG. 3C Pairs of Disc Electrophoresis runs of the blood sera of four different individuals showing normal genetic variability.

FIG. 3D The absorption spectrum of the anion of a dye that binds strongly to the cationic groups of acid-denatured protein. By measuring the “background” intensity,  $I_0$ , at wavelengths longer than  $610\text{ m}\mu$  and the intensity transmitted by the sample,  $I$ , with a narrow spectral band of green light, a reproducible “double-beam” technique is easily instrumented. Substitution of a narrow band violet filter for  $F_2$  permits measurement of discs of protein that have excessively high  $O.D.$  at the absorption peak.

of the morphological sciences usually have been constructed starting with the specific, and working down to the more general comprehensive class. But for the infant, learning probably starts with general classes within which he discovers special subclasses (e.g., see (4) and (21)). Can we, like the child, in our efforts to learn from observing this new universe, start at the trunk of a tree and work up to the branches? (See Turing's discussion of the "child machine" (3).)

#### EFFICIENCY AND TAXONOMIC CLASSIFICATION

Classification is, in fact, a naming procedure. It involves the subdivision of a population of objects, patterns, etc., into subgroups such that individual members of each subgroup are, on the basis of comparison of a set of corresponding attributes, more "similar" to one another than to members of other subgroups. If there are  $Q$  unrelated, equally frequent and dissimilar subgroups in a classification, then it will take an average of  $Q/2$  comparisons of an unidentified sample to match it to its proper subgroup and "name" it. The names (or code) of unrelated dissimilar subgroups constitute what is called a "non-significant code," that is, the code in no way indicates any relationship or degree of "similarity" of one subgroup to another (22).

When a classification takes the form of a taxonomic tree, members of subgroups (branchlets) of the same branch will, in general, be more "similar" to one another than to members of other branches. If there are, on an average,  $x$  levels of branching and  $y$  branches at each branch point, then  $Q \approx y^x$ , but it will take only an average of something like  $xy/2$  comparisons of an unidentified object to match it to its proper subgroup and "name" it. (If  $y = 2$  and  $x = 100$ , the identification of a sample by a taxonomic procedure would involve only 100 matching steps as compared to an average of  $2^{100}/2$  steps for the same number of nonsignificant pigeon-holes!) If the code for the name of each subgroup is derived from the "numbering" of the branches of the tree on which the subgroup is located, the code will be a "significant" code (22) and simple inspection of this "name" will indicate inter-group similarities. We are interested in the generation of a taxonomic classification and a "significant" code, (especially because a language which uses such a code may also be able to more efficiently convey "meanings" (see page 472)).

#### CORRESPONDENCE, IDENTITY AND SIMILARITY IN ONE AND TWO DIMENSIONS

In order to usefully compare patterns, it is necessary to design some measure or measures of the "similarity" of one pattern to another. All measures of similarity hinge on definitions of identity. Two objects (patterns, collections, properties, attributes, attribute sets, etc.) are said to be identical if, when "placed in a one to one correspondence," all corresponding parts are equal. For two-dimensional line figures—e.g., triangles, circles, etc.—two objects (occupying different positions in time-space) are said to be identical if, by a combination of translation and rotation they can be made "congruent". For two



dimensional patterns with varying optical densities, the patterns are said to be identical if for every point in one pattern there is a "geometrically corresponding" point in the other and vice-versa, and the optical density of each point is equal to that of its "corresponding" point. An equivalent of the "congruence test" (which provides an operational means for locating "corresponding" points) in this case, involves preparing a "perfect negative" (in the photographic sense\*) of one pattern, and, by rotation and translation, the negative and the second pattern are superimposed and "searched" until the minimum amount of light is transmitted through the total area of the superimposed patterns. If the measurements, at this minimum, of optical densities at all points of the superimposed patterns are equal to one another, and in addition, equal to the sum of the optical densities of the point of maximum density and the point of minimum density of the second pattern, then the patterns are identical and the points of the superimposed negative and positive have been placed "in correspondence" at this minimum.† In a similar manner, the concept of identity might be extended to three (or higher) dimensional patterns or objects.

#### SIMILARITY AS A DISTANCE BETWEEN PATTERNS

Whereas this definition of identity may be quite unambiguous, this is not the case for "similarity." It is helpful to represent the description of an object by a point in an  $n$ -dimensional space (hyper-space), where each attribute of an object (or position in a pattern) is considered to represent a separate "dimension" of the object, and the magnitude of each attribute represents its coordinate (distance from its appropriate coordinate axis) in that dimension of a hyper-space (e.g., see 25, 26, 27). When the problem of recognizing which attribute of one pattern corresponds to that of another is straightforward (as in the case of our one-dimensional electrophoretic patterns—we know the location of the "origin"—and for things such as lists of clinical data where each attribute is identified—e.g., white blood cell count, blood sugar, etc.) then it is easy to specify the total set of coordinates for each point in the particular hyper-space for the members of any group of objects with a corresponding set of attributes. If two objects (or patterns) are identical, they will have the same coordinates (i.e., they will occupy the same point in the particular hyper-

\* A "perfect negative" is defined as having an optical density for the point corresponding to the point of minimum density in the original, equal to that of the point of maximum optical density in the original, and each other point of the "negative" will have an optical density equal to that maximum optical density minus the optical density of the corresponding point in the original.

† It is of some interest that there is strong evidence that a simple diffusional "random search" permits the "discovery" of the appropriate positions of point to point correspondence between the extremely long ( $10^4$  to  $10^7$  bit (23)) messages of the complementary "one-dimensional" strands of DNA both in the *in vivo* pairing processes that occur in meiosis and somatic pairing as well as in non-living test-tube "nucleic acid hybridization" experiments (24). These experimental results suggest that a random search, using a measure of similarity (see next section) as a figure of merit, might be a surprisingly efficient device, especially if the search were begun at low resolution and finished up at high resolution.

space). If the hyper-space is an ordinary Euclidean metric space, the "distance" between the points in this space will be defined by the square root of the sum of the squares of the differences between the magnitudes of the corresponding attributes of a pair of objects (the  $n$ -dimensional version of the Pythagorean Theorem). The distance between identical objects will be zero. Any non-identical objects will be located at some "distance" from one another. The magnitude of the "Euclidean metric distance" defined above, has often been

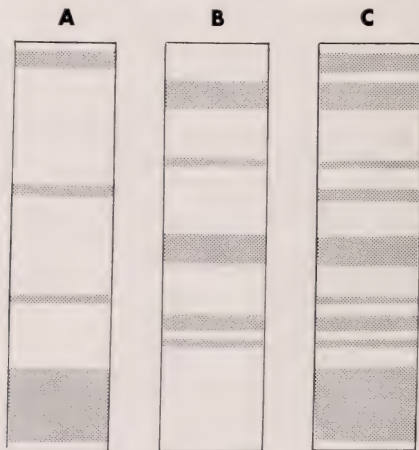


FIG. 4 Pattern *A*: low uniform optical density (0.01) over  $\frac{3}{4}$  of its area and optical density 1.0 over the other quarter. Pattern *B* also has  $\frac{3}{4}$  of its area almost completely and uniformly transparent but the points conjugate to those which absorb strongly in *A* here have the high transparency, while a different quarter of its area has an optical density of 1.0. Pattern *C* has the identical distribution of the high density regions of both *A* and *B* ( $\frac{1}{2}$  of its area) and has high transparency at all other points.

used as a measure of similarity, (e.g., see 28, 29, 30). Zero distance means identity, and infinite distance, ultimate dissimilarity.

Intuitively, this kind of definition of similarity—a "distance" between points in a hyper-space—is quite appealing. However, the use of a "metric space" where distances are defined as, for example, in a Euclidean space, often has certain intuitive as well as computational disadvantages. Consider the three patterns in Figure 4. Pattern *A* has very low uniform optical density (0.01) over  $\frac{3}{4}$  of its area and has a distribution of larger finite (1.0) densities over the other  $\frac{1}{4}$ . Pattern *B* also has  $\frac{3}{4}$  of its area almost completely and uniformly transparent but the points conjugate to those which absorb strongly in *A* here have the high transparency while a different  $\frac{1}{4}$  of its area has an optical density of 1.0. Pattern *C* has the identical distribution of the high optical den-



sity regions of both patterns  $A$  and  $B$  ( $\frac{1}{2}$  of its area) and has the high transparency at all other points.

In one intuitive sense,  $A$  and  $B$  are extremely dissimilar, but both are similar to  $C$ . Now the properties of a metric space are such that the distance between  $A$  and  $B$  can never be greater than the sum of the distances between  $A$  and  $C$ , and  $B$  and  $C$ . In fact,  $B$  will be only  $\sqrt{2}$  further from  $A$  than  $C$  in Euclidean metric space.

To satisfy our intuitive feelings about similarity for this test case, we must find some different measure (definition) of "distance"—one which will describe  $C$  as being "close" to both  $A$  and  $B$ , while still permitting  $A$  and  $B$  to be "very far" from one another.

#### METRIC AND SEMI-METRIC DISTANCES AS MEASURES OF SIMILARITY

Hyper-spaces are characterized by the kinds of expressions that are used to define the distances between points. The distance defined by the square root of the sum of the squares of the differences between the coordinates of two points characterizes a Euclidean metric space where if  $A$  and  $B$  are close to  $C$ , they can not be too distant from each other. A distance formula, using the  $n$  coordinates of each pattern, and which permits  $A$  and  $B$  to be close to  $C$  but far from each other (even infinitely far) defines a more general class of spaces, among which are an infinite number of so-called "semi-metric" spaces. In all such spaces, the distance between identical objects must be zero (as in a metric space—and indeed, all spaces), but the measures of similarity or dissimilarity may be very different from space to space. Clearly then, the choice of a measure of similarity must, in a sense, be arbitrary, but it is reasonable to examine common concepts of similarity to at least see if we can find a useful match between one or more arbitrary "distance" measures and our intuitive feeling for what we usually mean by similar or dissimilar (as in the case of patterns  $A$ ,  $B$ , and  $C$ ).

Dr. T. T. Tanimoto has proposed (e.g., 31, 32, 33) the following measure: Consider the number of equal pairs of corresponding attributes of two objects. Were these the complete set of attributes of the two objects, the objects would be identical. The presence of even one additional unequal pair of corresponding attributes would destroy the identity relationship, but would leave the objects "quite similar." More and more additional pairs of unequal attributes would "increase the distance" between these objects. Dr. Tanimoto proposed that the ratio of the number of pairs of non-zero identical corresponding attributes to the total number of pairs of corresponding attributes, of which at least one member is not zero, be defined as the "Similarity Coefficient." This would have a value of 1 for identical objects and 0 for extremely dissimilar objects and is similar to a probability (in this as well as other respects). A distance formula defining a particularly useful semi-metric space is the negative logarithm to the base 2 of the Tanimoto Similarity Coefficient and is a kind of measure of information (for further discussion see 31). This "Tanimoto distance" is zero for identical objects, increases for less similar objects, and is infinite for extremely

dissimilar ones, satisfying, in at least a gross way, our intuitive feelings for the properties of a reasonable measure of similarity.

The Similarity Coefficient was originally defined by Tanimoto for attributes which had magnitudes of either 0 or 1 (the binary case). He has extended it to cover the continuous case, and here the definition takes the simple form: Similarity Coefficient equals the sum of the smaller of the magnitudes of each pair of corresponding attributes (coordinates) of a pair of objects divided by the sum of the larger of the magnitudes of each pair of corresponding attributes of a pair of objects. For the purposes of illustration, our three patterns can be represented as 3 sets of 4 corresponding attributes: Pattern *A* may be represented as 1.0, 0.01, 0.01, 0.01; *B* as 0.01, 0.01, 1.0, 0.01; and *C* as 1.0, 0.01, 1.0, 0.01.

If we examine this Similarity Coefficient among patterns *A*, *B*, and *C*, we find that the similarity between *A* and *B* is very near 0 (0.02), but the similarity of *A* or *B* to *C* is 0.51. The ratio of the corresponding Tanimoto distances is 5.7 for this case where the transparent background has a non-zero (0.01) optical density. If the background were completely transparent (optical density zero), the ratio of the two distances would be infinite in "Tanimoto Space." In Euclidean metric space, however, the ratio of the two distances would still be  $\sqrt{2}$ . The Similarity Coefficient between *A* or *B* and *C* would equal 0.50 with a completely transparent background. Clearly, "Tanimoto Space" provides a measure of similarity, at least with this model, that is very much closer to our intuitive concepts of similarity and dissimilarity. The Similarity Coefficient is also simpler (therefore cheaper) to compute than a Euclidean metric distance function.

Many other measures of similarity can be devised, some with very special characteristics, such as the classical Correlation Coefficient,

$$C_{xy} = \frac{\sum(\bar{x} - x_i)(\bar{y} - y_i)}{\sqrt{\sum(\bar{x} - x_i)^2 \sum(\bar{y} - y_i)^2}}.$$

The Correlation Coefficient varies from +1 to -1. Objects which are identical have a Correlation Coefficient of +1 (with the restriction that the Correlation Coefficient between identical or non-identical *uniform* objects—i.e., values of all attributes of an object equal to each other—is indeterminate). Objects which would be identical to each other if all the attributes of one were either multiplied by a single appropriate constant and/or a constant were added to all the attributes of one, also have a Correlation Coefficient of +1. Objects with Correlation Coefficients of -1 are either perfect "negatives" of one another (magnitudes of corresponding attributes vary exactly inversely) or would be perfect negatives if all the attributes of one were multiplied by a single appropriate constant and/or a constant were added to the attributes of one. (In one sense, pairs of objects with a Correlation Coefficient of -1 are the most dissimilar of objects.) To summarize, the Correlation Coefficient gives a measure of similarity, normalizes for proportional and/or additive differences, and provides a measure of the "symmetry of opposites" by so-called "negative correlation." For our test patterns, the Correlation Coefficient between *A* and *B* is

-0.333, and between *A* and *C* or *B* and *C* is +0.577 (and these remain the same if the background is made completely transparent).

This measure of similarity also has a kind of intuitive appeal—but different from that of Tanimoto's Similarity Coefficient. To convert the Correlation Coefficient to a distance measure for defining another semi-metric space, we can again take the negative logarithm. (The logarithm of  $-1$ , like the  $\sqrt{-1}$ , is an imaginary number. Thus "Correlation Space" includes two spaces which share their "points at infinity" in common. One is a real semi-metric space and the other is an imaginary space with a corresponding imaginary point for each point in the real space.)

The Correlation Coefficient is a very powerful tool for recognizing certain classes of similarity which might otherwise go unnoticed without an additive or multiplicative normalizing step. However, in cases where the Correlation Coefficient is  $+1$  as a result of compensating for both additive and multiplicative differences simultaneously, this result will usually appear to be in complete conflict with an ordinary intuitive sense of similarity or identity; (for example, consider as paired objects, the two sequences of numbers, 9, 6, 12, 3, 15, and 9, 8, 10, 7, 11). It is also, incidentally, about twice as expensive to compute the Correlation Coefficient as the Tanimoto Similarity Coefficient. On the other hand, two patterns in which the magnitudes of the attributes vary independently and at random with respect to one another, will have a Correlation Coefficient of 0 and will be infinitely separated in Correlation Space. This is clearly an attractive feature. Such a pair might occasionally be as close together as one Tanimoto unit in Tanimoto Space (see page 461).

No other measure of similarity of which we are aware seems to approach the Tanimoto Similarity Coefficient or the Correlation Coefficient in both generality and sensitivity, although simple theoretical arguments lead one to believe that there must be a host of as yet undiscovered but useful measures of similarity (distance functions of both metric and semi-metric spaces).

#### REWEIGHTING AND NORMALIZATION

Two kinds of transformations can be usefully performed on data before measuring the similarity of patterns. These constitute reweighting the data based on the informational content (statistical properties) of the data (e.g., removing redundant data), and normalizing the data—i.e., performing transformations such that two patterns, which before transformation might be quite far apart in a given semi-metric space, will, after transformation, "superimpose" (be identical). Simple transformation may involve translation or biasing (modifying the position or magnitudes of the attributes by an additive constant), expansion or contraction of scale (modifying the magnitudes of the attributes by a multiplicative constant), forming the "negative," mirror inversion, etc. The purposes of reweighting and normalizing are different although operationally they may be indistinguishable. Thus the removal of redundant information by dividing all values of an attribute of a population by the lowest common denominator among the values of that particular attribute

among the members of that population—one kind of reweighting step—is indistinguishable from a normalizing contraction of scale in the dimension represented by that attribute.

All normalizing or reweighting steps must be of such a nature that when applied to initially identical patterns, the distance between such patterns will remain zero after the normalizing or reweighting procedure. It is also desirable that normalizing or reweighting steps should be of such a nature that no useful information about differences be lost or discarded inadvertently during data processing.

We further distinguish these two processes as follows:

### *Reweighting; the Removal of Redundancy*

The general purpose of reweighting is to reduce the magnitude of a special kind of similarity (e.g., redundancy) in a given population. This is done to increase the sensitivity of a similarity measure for detecting the remaining differences between patterns. It is desirable that reweighting reflect the statistical significance (or information content) of the raw data. Because of its very nature, inadvertent loss of useful information during reweighting is unlikely.

### *Normalization; a Topological Transformation*

The general purpose of normalizing is to increase the sensitivity of a similarity measure to otherwise "hidden similarities" between patterns. Now just what do we mean by "hidden similarities"? Two samples of a single volume of serum, one diluted to half the concentration of the other, would give disc electrophoresis patterns in which the optical density at each band of the undiluted specimen would be twice that of the corresponding band in the other (see Fig. 5). If we arbitrarily decide that such a uniform dilution has little significance for our purposes, but the fact of identity after a change of scale is highly significant, then the application of this transformation is called a normalizing step. Clearly, if we are mistaken about the relative significance of the difference in concentration as compared to the "hidden identity" of these samples, and if we employ a normalizing step which "throws out" the original information about the difference in concentration, not only will we fail to discover our error from the ensuing analysis, but we could never retrace our steps.

Normalization can therefore be hazardous. To eliminate this hazard it might be desirable to compare raw data without any normalization and then to again compare the same data after normalization. If we are in complete ignorance about the information content of the universe of the population being sampled and classified, there is no doubt that this safe procedure is the only reasonable procedure (even though it is also a more laborious one).

When, on the other hand, one has chosen or designed the straight-jacket into which the "raw data" must fit—the particular method of measurement or observation or environmental transducer or "sense organ" used—one has usually already passed the rawest data through a variable number of implicit or explicit normalizing steps. From this frame of reference therefore, when

economy is important, it is more realistic to try to recognize such data-gathering normalizations explicitly, and to add any others which were not conveniently included in the data-gathering process but can be argued to be "reasonable." If the data are also preserved in something close to their original form, as well as in the normalized forms, it will always be possible to reexamine those original differences between samples which disappear after normalization.

As indicated earlier (page 450), the Correlation Coefficient can automatically provide two kinds of normalization while simultaneously providing a measure of similarity. It is largely because of an "over-abundance" of kinds of

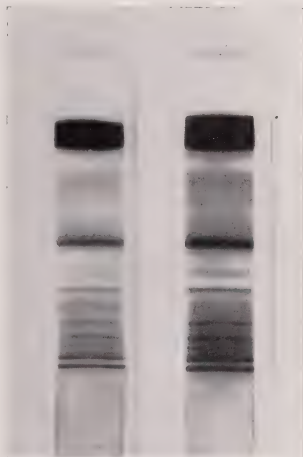


FIG. 5. One of the samples from Figure 3C: 3 microliters of serum in the right-hand run, 1½ microliters in the left-hand run.

normalization that I consider it inappropriate (hazardous) as a primary measure of similarity.

In general, normalizing steps should usually precede reweighting steps because it is the redundancy of the normalized population that one wishes to reduce.

#### A SIMPLE TAXONOMIC SCHEME FOR ONE-DIMENSIONAL PATTERNS

We will now describe a scheme of analysis starting with a matrix of 10,000 columns (the samples) and 400 rows (the positions in each pattern) with 128 numbers from 0 to 1,000 (the anti-logs of the 7 bit logarithms of concentration, accurate to 5 binary places) occupying the positions in the matrix.

Given such a matrix, we now apply a normalizing step to insure that samples which are identical, except for a dilution of one relative to the other, will



be equal. This is accomplished by dividing the value of each attribute in a column by the average value for that column.

Next comes a reweighting step. If we locate the smallest non-zero value in each row of the normalized matrix and divide each value in that row by that smallest value, entering such quotients in a new conjugate matrix, we will have reweighted the data so that all corresponding attribute magnitudes shared by all samples will have been removed. With this redundant information eliminated, the measurement of similarity between patterns (columns) will be more sensitive to the magnitudes of residual differences from pattern to pattern. (It is clear that a good deal of sharing of attributes will usually still remain to decrease the sensitivity to differences.) The above procedure is the simplest\* for increasing sensitivity to differences without losing information.

The Tanimoto distances between each column and every other column are now computed and are entered into a new matrix, the Similarity Matrix, with 10,000 rows and 10,000 columns. The average Tanimoto distance between each sample and every other sample, i.e., the sum of the entries along a row of the Similarity Matrix divided by 10,000, is computed and samples are ordered in a list on the basis of this average Tanimoto distance. The sample with the smallest average distance from all other samples, (i.e., the "most typical" member of the population) will head this "Hierarchical List" (31).

A histogram of the frequencies of the distances between this most typical member and all other members is plotted. The first "peak" is located. (This relative maximum might rarely occur at the origin.) If it falls more than 1 Tanimoto unit of distance (Similarity Coefficient less than  $\frac{1}{2}$ ) from the origin, all specimens within 1 Tanimoto unit of the most typical specimen (as well as the most typical specimen) are transferred to the bottom of the Hierarchical List. The sample now heading the List is selected as "a most typical member" and a histogram of the above type is again plotted, and its first peak located. If the peak falls more than 1 unit of Tanimoto distance from the origin, the Hierarchical List is again rearranged.

If, on the other hand, a first peak is within 1 unit of Tanimoto distance of a most typical member, the first minimum in the distribution curve with a magnitude less than  $\frac{1}{2}$  of the value of the first peak is located (point A) (see Fig. 6). The next point towards the origin with a magnitude equal to  $\frac{1}{2}$  the value of

\* A more balanced reweighting requires more sophisticated data processing. For example, the number of occurrences of each normalized value in each row divided by 10,000 gives us the frequency of occurrence of that normalized concentration of protein at that position in the pattern,  $(f(x))_r$  = frequency of a particular value of  $x$  at position (row)  $r$ ). In so far as the samples making up the set of 10,000 were chosen at random from a given larger population, the set of frequencies approximate the probabilities that a new sample, picked at random from that larger population will have each of these normalized concentrations at each position ( $p(x)_r$  = probability of a particular value of  $x$  at position  $r$ ). By multiplying each entry,  $x$ , by  $-\log_2 p(x)_r$ , (the self information (22) of each kind of entry in a row), the magnitude of very rare values of the attributes are emphasized, independent of their original magnitudes. This method of reweighting will be used further on in a more sophisticated scheme.

the first peak is located (point *B*). Then the next point towards the origin with a magnitude equal to  $\frac{3}{4}$  of the value of the first peak is also located (point *C*). Twice the distance along the abscissa between the points *B* and *C* is added to the value of the abscissa at *B* to locate a new point on the abscissa (point *D*). The "Boundary of the First Branch" is set by either the location of the minimum, *A*, or the point *D*, whichever is further along the abscissa from the origin. (In this procedure, *D* is the intersection with the abscissa of the line drawn through points *B* and *C*. If the distribution function of the marginal portion of this sub-group were Gaussian, less than 4.5% of the "true" members of that group would lie at Tanimoto distances greater than *D* from the most typical member.)

All specimens within the "radius" (*D* or *A*) of the "semi-metric space hypersphere" surrounding the most typical member or "Archetype" constitute the

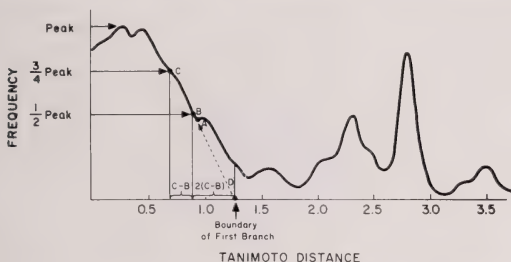


FIG. 6. Model of a frequency distribution (histogram) of Tanimoto distances between a "most typical" sample and all other samples, demonstrating the application of the rules for determining the boundary of the First Branch population.

membership of the First Branch population and are entered into a new matrix, the First Branch Matrix.

All members of the First Branch within 1 Tanimoto unit of the Archetype are removed from the Hierarchical List and the highest remaining member on the list is chosen as a candidate for the title, Archetype of the Second Branch. All the steps performed with a most typical sample are repeated with this sample to locate the boundary and members of the Second Branch (which may also include some marginal members of the First Branch). These samples are entered into a Second Branch Matrix, and appropriate members of this Branch are removed from the Hierarchical List, and the whole process is iterated until less than 1,000 members remain on the List. All these remaining samples are then added to the "Residue Branch Matrix of the Trunk."

The numbers of members in each of the Branch Matrices is noted and the Branches are ordered according to their sizes.

A new sample (the ten thousand and first) is now normalized and re-weighted with the set of lowest common denominators of the rows from the



original Trunk Matrix (as were all the original samples) and is compared to the Archetype of the Largest Branch (other than the Residue). If the Tanimoto distance between them is less than the radius for that Branch, the sample is added to that Branch Matrix. If the distance also is equal to or exceeds 1 Tanimoto unit, or if it exceeds the radius of that Branch, it is next compared to the Archetype of the Next Largest Branch, and the entire process is iterated until a Branch is located, the Archetype of which is closer than 1 Tanimoto unit to the new sample, or failing that, the new sample is added to the Residue Branch Matrix.

This procedure is repeated with succeeding new samples until the population in a Branch Matrix equals 10,000. At that time, the Branch is normalized, re-weighted, and subdivided in exactly the same manner as the original Trunk population. This procedure generates a taxonomic tree and at the same time, provides a means for locating those sets of samples out of the total population of samples studied which are most like a new case. (By examining other attributes (e.g., clinical findings) from the records of the set of patients in a "terminal" Branch, and adding these additional attributes—rows (attributes) may be compared and classified in the same way that patients' patterns (columns) were compared above, and if the pattern characteristic of a "terminal" Branch is diagnostic of a particular disease entity, then the most significant attributes of the pattern will be grouped with the constellation of clinical findings that are known to characterize the disease.)

#### JUSTIFICATION OF THE SCHEME

Let us now examine the justification for these steps explicitly, and discuss the amount of computer time required for such data processing. We will then examine a more sophisticated technique which has grown from the above procedure. This technique drastically reduces cost (by a factor of 1,000 or more), reduces "arbitrariness," and increases flexibility and generality. The refined technique will be listed in a somewhat more formal way, as a set of instructions from which a programmer could more easily and directly work.

#### GROUP CENTROID, "NATURAL" BOUNDARIES BETWEEN GROUPS, AND HYPER-SPHERES

When one considers subdividing a group of objects, patterns, etc., to generate a taxonomic tree (classification), one usually assumes that some cohesive clusters with easily definable "natural" boundaries exist within the population to be subdivided. This assumption is not necessarily valid. Consider a population of patterns in which the optical densities have a Gaussian density distribution of amplitudes that vary at random from point to point within a pattern and from pattern to pattern. Such a "white noise" source has no "internal structure." If we examine the distribution in a hyper-space of the points represented by such a group of patterns, we will discover a single cluster with a "bell-shaped" density distribution (unimodal) from its "center" out, and no subdivision of such a population would yield "natural subgroups" (Fig. 7).

Consider next, a population of  $m$  discrete kinds of patterns which are as distinct as patterns  $A$  and  $B$  (page 448). Such a population would provide a set of  $m$  discrete points in a hyper-space. Now superimpose low magnitude "white noise" on the individual samples of discrete patterns and examine the distribution of the new noisy patterns. If the magnitude of the noise, relative to the pattern optical densities, is not too great, we will now have a polymodal distribution of  $m$  distinguishable clouds or clusters, each centered on the position of the corresponding discrete point in the previous distribution (Fig. 8). If we

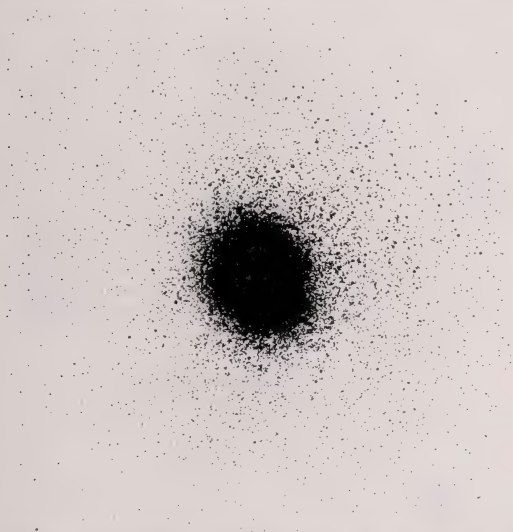


FIG. 7. Model of the distribution of points representing members of a "white noise" population of patterns in a two-dimensional metric space.

increase the magnitude of the noise, these clouds will overlap—and if the signal to noise ratio degenerates sufficiently, the overlapping clouds may become indistinguishable from a single "white noise" distribution.

When there is no "overlap" of clouds, a very large number of kinds of hyper-surfaces (boundaries between  $n$ -dimensional volumes) can be generated in the hyper-space, any set of which will sub-divide the population into the same set of  $m$  subgroups. Such subgroups will be called natural subgroups. However, as soon as we have "overlap" between two such "noisy" populations, there exist many fewer kinds of surfaces which will yield identically divided populations. Any dividing hyper-surface is then arbitrary, and any claim to

"naturalness" for a classification derived from such data can receive its only support from the arguments used to defend the particular boundary-defining conditions and distance function used.

It is convenient to consider the "spherical" surface of the hyper-sphere (surface generated using the distance from the "center of mass" or centroid of

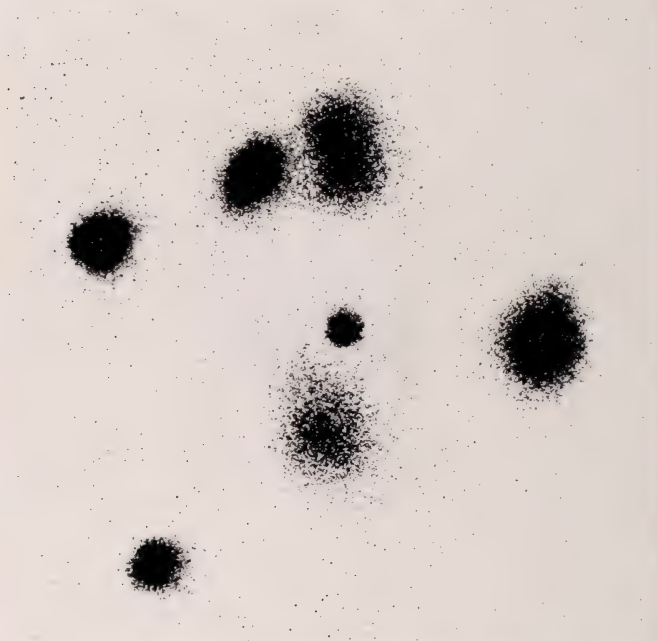


FIG. 8. Model of a polymodal distribution of  $m = 7$  different "noisy" populations of patterns in a two-dimensional metric space.

an ideal natural cluster to its most distant member as the "radius") as the boundary of the cluster (31, 34, 35). (Of course, for the natural subgroup, many other arbitrary hyper-surfaces can usually serve as well, but no other is as conveniently defined. Some infrequently encountered but none the less distinct "natural" populations could never be resolved by a simply connected surface such as a sphere—e.g., two interlinked toroids. To discover and separate such populations, less convenient algorithms which search for discontinuities in connectivity must be used.)

Operationally, to generate such a hyper-spherical boundary surface for natural or unnatural clusters, we need two devices:

- 1) We must locate the centroid or a reasonable substitute "central point."
- 2) We must find a radius measured from that "central point" which generates a hyper-sphere enclosing most of the members of an unnatural cluster, excluding most members of other clusters, and hopefully subdivides most natural populations without any admixture.

#### RELATIVITY IN METRIC AND SEMI-METRIC HYPER-SPACES

In Euclidean space, if we seat ourselves at any convenient point in the co-ordinate system and view the relative distances between galaxy clusters, galaxies, star clusters within a galaxy, and individual stars, we can then move to another observation point, and the relative distances between points in this space will be observed to be the same (assuming the use of some rangefinder with infinite resolution). A procedure for locating clusters operating from the origin of the co-ordinate system, or any other fixed point could therefore serve the requirements of device 1). Unfortunately, as pointed out earlier in the discussion of metric spaces, points in  $n$ -dimensional Euclidean space will often be quite "crowded" and overlap of subgroup spheres would therefore be expected to make natural subdivision quite rare.

On the other hand, in a semi-metric space such as Tanimoto Space, where cleaner separation of dissimilar clusters can be anticipated, the universe and its galaxies present very different relative views, depending upon the point in space from which one makes his observations. From point  $C$ ,  $A$  and  $B$  are both relatively and equally nearby, yet from  $A$ ,  $B$  is very much further away than nearby  $C$ .

To locate the centroid of a cluster in a semi-metric space, it is therefore necessary, in principle, to sit down at each and every point in that space and look outwards at all other points, and on that basis locate the centroid of a cluster.

#### CLASS ARCHETYPE AND THE HIERARCHICAL LIST

If a cluster is "unimodal" and "symmetrical," the centroid will be closest to the "most typical" member of the population, and the average Tanimoto distance from this member to other members of the cluster will have the minimum value. That is, among the average Tanimoto distances between each member of this cluster and every other member, the "most typical member" will have the lowest value and will therefore head the Hierarchical List (see Tanimoto, 31).

If the cluster is not unimodal—for example, is polymodal, with the centroid of each of the subgroups distributed equally spaced on a "spherical shell," then the point at the "center" of the shell will be the centroid for this polymodal population. Let us suppose that the spread of each cluster is sufficiently large to extend to the center of the sphere (Figure 9). Then the specimen nearest the center will head the Hierarchical List for this polymodal population.

In the case of the unimodal population, the "most typical" member will have a larger number of closest neighbors than any other member. In contrast, in the case of this spherical shell polymodal population, the member nearest the centroid will have among the fewest number of closest neighbors. If the shell radius is a great number of Tanimoto units in magnitude, then the

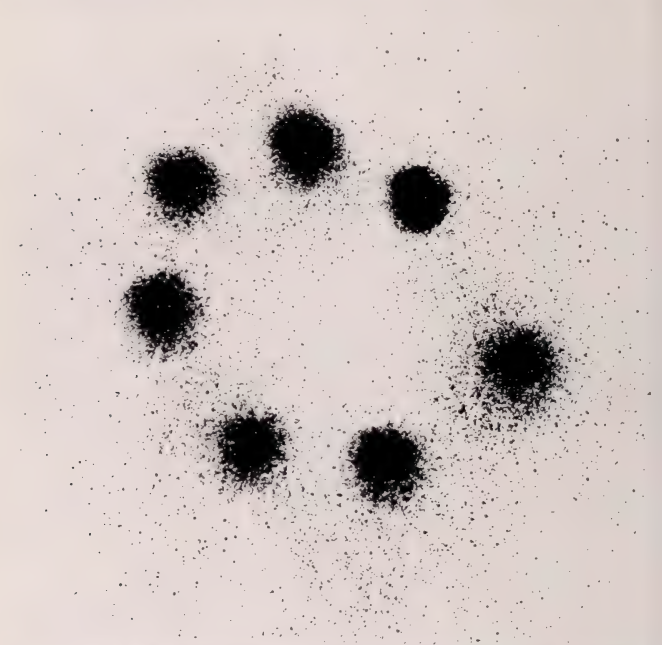


FIG. 9. Model of a "spherical shell-distribution" of 7 "noisy" populations of patterns in a two-dimensional metric space.

specimen nearest the centroid will really be a very poor Archetype. Such a polymodal subgroup may none the less be quite natural in being completely separated from other more distant subgroups (unimodal or polymodal).

If we wish the "Archetype" to have the connotation of a "most typical" sample, we must reject such samples near low density centroids as candidates for the title, Archetype of a Branch. Alternatively—and with somewhat less arbitrariness—we might use the designation, Population Centroid, understanding that such a member of a population may be a far cry from what one intuit-

tively thinks of as a "type specimen" or Archetype. (The two approaches will yield somewhat different branching patterns—(i.e., they will yield different intermediate subgroups) although the number and identity of the "terminal" branches of two such taxonomic trees would be expected to be quite similar.)

The Hierarchical List provides us with the "Population Centroid" of the Trunk. Examination of the frequency distribution of Tanimoto distances between the Population Centroid and all other samples tells us whether it is located in a low density or high density volume of Tanimoto Space. The magnitude of this distribution function and the sign and magnitudes of its first and second derivatives provide all the necessary cues. Clearly, however, the boundary between what one considers to be low density and high density must be arbitrary. We have above proposed using the kinds of criteria that are commonly used in analysis of histograms. (There is obviously considerable room here for the use of more sophisticated criteria which, for example, examine boundaries using assumptions of other than normal distribution (36, 37).)

The following special property of the Similarity Coefficient was also taken into account:

It can be shown that the average Tanimoto distances between a sample of a population, in which all magnitudes of all attributes are equally likely, and all other members have a distribution with the mean location of a peak at 1 Tanimoto unit, and approaches minimum values at both zero and infinite Tanimoto distances. Therefore patterns related only randomly might average separations as small as 1 Tanimoto unit (as will also such clearly related patterns as for example, A and C).

#### SUBDIVISION OF THE POPULATION

By transferring a Population Centroid and all its neighbors within 1 Tanimoto unit to the bottom of the Hierarchical List when the first peak is located beyond 1 Tanimoto unit (as would be the case for the polymodal shell model distribution, following the analytical scheme proposed on page 454) we make it more likely that the next highest remaining member on the Hierarchical List will fall near a high density point which is also near a centroid. Transfer of only the centroid itself would usually leave the next nearest member to the centroid at the top of the Hierarchical List. By transferring a sufficient number of closest neighbors of the centroid, we therefore insure a shift to a new center of gravity. In the shell model, this new "central point" would be near the center of one of the overlapping subpopulations on the shell, and the first minimum in the distribution curve would occur beyond the furthest subpopulation on the "opposite side" of the shell, if the populations overlapped sufficiently. If not, an individual subpopulation would be isolated including some marginal members of neighboring subpopulations and excluding some (of the order of 4.5%) of the "proper members" of that first subpopulation.

By using either a Population Centroid, or an Archetype as defined above, we thus locate useful "central points" satisfying the requirements for device 1).



The simple rules for determining the group radii are given on pages 454 and 455 satisfy the requirements for device 2).

Such simple rules can result in "too early" subdivision of a natural subgroup—i.e., two overlapping subgroups may have a finite minimum between them but a zero minimum beyond the second. A set of rules for more natural subdivision would call for preliminary search for the widest or "most significant," minimum with rules for deciding whether or not to use its location, or that of the first minimum (or any other minimum closer to the origin) as the basis for setting the radius for the subgroup.

The kind of boundary rules described assure that marginal members of groups will appear in more than one Branch of the Tree. This partly compensates for the arbitrariness of "unnatural" boundaries, but does this at the expense of increased computational cost.

Some elements of the analysis presented, though intuitively simple, are none the less relatively novel. We have attempted to resolve the classification problem with methods which have their origins in ordinary intuitions, not because we believe that intuitively appealing methods are necessarily the best, but because experience has often shown that if new and useful methods are to be discovered during an essentially exploratory phase of the development of a field, aimless groping for new directions often results from the loss of perspective that can follow when one casts loose, too early, from intuitive moorings.

Whereas the boundaries of our populations, i.e., hyper-spherical surfaces, are set by a semi-metric distance (such boundaries are now usually referred to as *decision boundaries* in the jargon of decision theory, a new branch of statistics (38, 39) which threatens to engulf the whole), it is more commonly the practice, in decision theory, to carve up a metric decision space with hyper-planes. Less commonly, hyper-spheres and hyper-quadrics have been suggested as promising alternatives (35). The implementation of such techniques will usually be more complicated than the calculation of a simple semi-metric radius, and the complexity of the computations will usually grow very rapidly as one progresses from relatively few simple hyper-planes to the complex but less arbitrary hyper-quadric surfaces.

Because of the "asymmetries" of many interesting semi-metric spaces (including Tanimoto Space and Correlation Space) the simply computed semi-metric hyper-spherical boundary can often be expected to give very clean separation of "natural" populations which are themselves asymmetric in a metric space and/or are so crowded that only very complex decision boundaries could hope to separate them cleanly. Thus the use of a simple semi-metric space, in a sense, permits the equivalent of a topological distortion and separation of such natural populations sufficient to permit the use of very easily defined and simply implemented decision boundaries. This results from reversing priorities in setting up the problem. Rather than using a familiar metric space to define a less familiar measure of similarity, a more intuitive measure of similarity is used to define our semi-metric decision space. Tanimoto's Hierarchical List then permits the simple and unambiguous location of



those population centroids in semi-metric space which are necessary to generate the hyper-sphere—but at some expense. We will now examine this added cost to see whether the potential computational simplicity of this semi-metric approach can be preserved while reducing the cost to reasonable levels and at the sacrifice of no more than a modicum of added ambiguity.

#### COMPUTING COST

If any one kind of operation in such a scheme requires a number of magnitudes more computer-time than all the rest of the operations, examination of the economies of this operation will, in fact, give a reasonable measure of the total cost.

The construction of the Hierarchical List requires the computation of a number of Similarity Coefficients equal to one half the square of the number of samples in the List. The time to compute one Similarity Coefficient between two 400 position samples with 7 bit resolution at each position on the IBM 7090 is about  $25 \times 10^{-3}$  seconds (40). The computation of the Trunk Similarity Matrix (or any other Branch Matrix of  $10^4$  samples) would therefore require  $0.5 \times 10^8 \times 25 \times 10^{-3}$  seconds, or 14.5 days. The computer time per sample would average about two minutes, or at \$500 per hour, about \$17 per sample. Since, as one proceeds up the tree, appreciable numbers of samples will appear in more than one Branch, the cost might rise to a few times this figure.

Since, from the perspective developed to this point in the discussion, the "education" of the computer would require the growth of many levels of branches before the Tree might be diagnostically useful, the investment in computer time might have to become of moon-shot proportions before its utility could be tested. If the number of ultimate Branch Tips is fixed (the population has a finite number of natural indivisible subgroups such that, after addition of members in excess of the number necessary to activate the "subdivide instruction", all distribution histograms of Tanimoto distances among members of a Branch Tip remain unimodal and drop to zero beyond the peak) then beyond the time when those terminal Branch Tips have been resolved, the "education" of the computer would be complete. From then on, the time required for classifying each additional sample would drop appreciably (for example, for a symmetrical dichotomous tree with  $2^{30}$  Branch Tips, from two minutes per sample to 0.75 seconds). Thus, if one had great confidence in the utility of such a program—sufficient confidence to invest billions of dollars in the education of the computer before it "grew to a productive age," one might consider going ahead, even with such an enormous initial investment, since the ultimate cost per sample would be quite low.

We did not have *such* confidence. Therefore, it was necessary to examine ways for at least approaching the theoretical performance of such a system by means which would reduce the cost by a number of orders of magnitude.

Since the cost is proportional to the square of the number of samples in a Similarity Matrix, a reduction of the subdivision limit from 10,000 to 1,000 samples would result in a hundred-fold saving. Our original reason for arbi-

trarily specifying 10,000 samples per matrix was to try to assure that the lowest common denominators used for reweighting would be representative of the population as a whole with a very high degree of confidence. A more reasonable way to specify when a population should be subdivided would depend upon the observed statistical structure of that population such as might be revealed by continuous monitoring of the parameters of the part of the population sampled. In this way an explicit cue could be automatically provided when the particular population might be subdivided with a specified degree of confidence. If this device would lead to smaller Similarity Matrices, some economy might thereby be achieved. Alternatively, this less arbitrary device might lead to even larger matrices.

#### HISTOGRAM "CROSECTIONS" OF A SEMI-METRIC SPACE AND BYPASSING OF THE HIERARCHICAL LIST

More significantly, any device that might bypass the calculation of the Similarity Matrices and Hierarchical List to locate the "central points" of subgroups in Tanimoto Space would permit very substantial savings.

Consider a sample chosen at random from a population of 10,000 patterns. If we compute the Tanimoto distance between it and all other samples and plot a histogram of frequency versus distance, we will, in general, find a distribution curve with a number of maxima and minima. If we search out the highest peak in this histogram we will have located the largest number of specimens at any fixed distance from this first sample. These may either be all quite similar to one another, or as disjoint as our optical density patterns, *A* and *B*, (since patterns infinitely distant from one another may lie at finite and occasionally equal distances from a third pattern in a semi-metric space).

If we use the first histogram to locate a new specimen from this modal peak (any specimen at the distance of the peak from the first specimen), compute the Tanimoto distance between it and all other members and then plot a new histogram, one of the following distributions will be observed:

a) The distribution now peaks very near the origin. This peak is also the largest of the peaks in the histogram and includes approximately the same number of specimens as were included under the peak in the first histogram from which this sample was extracted. This would usually mean that the first peak in fact represents a unimodal and symmetrical group of specimens which were close to one another as well as at nearly equal distances from the first specimen, and this second specimen is therefore very near the "central point" of this largest First Branch population. The process may be repeated and after each iteration, the largest peak should move closer and closer to the origin and each newly extracted modal specimen should approach closer and closer to the specimen which is the "True Population Archetype." For the purposes of useful subdivision a close neighbor of the "True Archetype" will obviously be satisfactory and the degree of closeness required can be explicitly specified. If only ten such sequential "crossections of this Tanimoto Space" proved to be adequate to locate an "acceptable" Archetype, then the cost of subdivision would

have been reduced about 1,000 times. As the Taxonomy grows, the cost per sample rises to the ultimate cost per sample (of the order of 15¢ on the IBM 7090) with the "fully educated computer" above. (Such a cost is at least tolerable for a useful diagnostic tool.)

b) The distribution may now peak near the origin, but some new peak, far removed from the origin is instead the largest peak. This means that the peak in the first histogram was composed of two or more groups (e.g., like *A* and *B*) which have been separated by choosing one of their members as our new frame of reference. We now proceed to select a sample from the new largest peak and iterate. It will then either provide us with observations a) or b) above, or c).

c) The distribution now provides a set of two or more equal peaks of maximum height. The significance is the same as in b) and our strategy is also the same with any of the equal peaks being used for locating a next candidate for Archetype of the Branch.

Following observations b) or c), a larger number of crossections will in general be required to locate an "acceptable" Archetype for the Branch, but should still be orders of magnitude smaller than for computing an entire Similarity Matrix. The definition of "largest peak" is obviously open to some refinement which would, for example, take both "area under the curve" as well as peak amplitude into account.

This heuristic (41, p. 6) method of sampling the population with trial "crossections" through a semi-metric space to locate the candidates for Archetype of a Branch promises to provide the requisite economy to make this kind of Taxonomic procedure both practical at this time and the leading contender as a model for mechanical induction.

#### A MORE EFFICIENT PATTERN RECOGNITION PROGRAM

We will now list the steps of an improved Taxonomic procedure.

##### *Definitions*

$x_{rc}$  is the magnitude (to 5 significant binary places) of the anti-log of the 7 bit logarithm of the optical density (therefore proportional to the concentration of protein) at position (row)  $r$  in sample (column)  $c$ .

$n_c$  is the number of samples (columns) in an active memory matrix.

$n_r$  is the number of positions (rows) in a sample, (e.g., 400 in this case).

Then the row and column means are given by,

$$\bar{x}_r = \frac{\sum_c x_{rc}}{n_c}; \quad \bar{x}_c = \frac{\sum_r x_{rc}}{n_r}$$

and the matrix mean by,

$$\bar{x} = \frac{\sum_r \sum_c x_{rc}}{n_c n_r} = \frac{\sum_r \bar{x}_r}{n_r},$$

and a "conservatively biased" estimate of the standard deviation of a row is given by,

$$s_r = \left[ \frac{\sum_i x_{ri}^2}{(n_r - 1)} - \bar{x}_r^2 \right]^{1/2} \approx \left[ \frac{n}{(n - 1)} \right]^{1/2} \left[ \frac{\sum_i x_i^2}{n} - \bar{x}^2 \right]^{1/2} \\ \approx \left[ \frac{\sum_i x_i^2}{n} - \bar{x}^2 \right]^{1/2} \quad \text{for } n_c \gg 1.$$

The "Signal to Noise Ratio" for a row, which may be used as a *measure of confidence*† in the significance of the value,  $\bar{x}_r$ , is defined as,

$$(S/N)_r = (N/S)^{-1} = \frac{\bar{x}_r (n - 1)}{s_r} \approx \frac{\bar{x}_r n}{s_r} \quad \text{for } n_c \gg 1.$$

A weighted root-mean-square value of the  $(S/N)_r$ 's is given by,

$$(S/N) = n \left[ \sum (N/S)^{-2} \right]^{-1/2}.$$

All boundary defining steps which explicitly or implicitly affect the level of confidence by setting a limit of acceptable error or acceptable Signal to Noise Ratio will be marked with an asterisk (\*). The level of confidence considered acceptable is clearly arbitrary and may be modified from the values suggested below to either decrease or increase the overall confidence that may be placed in the "prediction"

† If, for each row, the magnitudes of  $x_{ri}$  are independent random variables, then from Tchebysheff's inequality (42), it can be easily shown that the probability that the limiting value of  $\bar{x}_r$  (the value of  $\bar{x}_r$  for  $n_c = \infty$ ) will differ from  $\bar{x}$  by less than  $\epsilon > 0$ , (where  $\bar{x}_r \pm \epsilon$  is the *confidence interval*) is

$$P_c > 1 - t^{-2},$$

where  $t = (\epsilon/\bar{x}_r) (S/N)_r$ ,  $(\epsilon/\bar{x}_r)$  is the maximum tolerable "Fractional Error" and  $(S/N)_r$  is the Signal to Noise Ratio as defined above.

If, for each row, the magnitudes of  $x_{ri}$  in addition have a unimodal normal density distribution over the columns, then from the Central Limit Theorem (42), for large  $n_c$ ,

$$P = \frac{1}{\sqrt{2\pi}} \int_{-\infty}^{\infty} e^{-t^2/2} dt,$$

which is the area under the normal density curve of mean 0 and standard deviation 1, for the confidence interval,  $\bar{x}_r \pm \epsilon$ . For  $t = 1.96$ ,  $P_c = 0.95$ ;  $t = 2.58$ ,  $P_c = 0.99$ ; and  $t = 3.30$ ,  $P_c = 0.999$ . (For small  $n_c$ ,  $t$  will be distributed approximately according to "Student's" distribution (43).)

If the distribution is actually multimodal, the probability,  $P_c$ , will generally be even larger. It will always be larger for multimodal distributions in which the component unimodal distributions are of Pearson type II (36) which includes the normal density (or Gaussian) distribution and the uniform (or "rectangular") distribution as the two opposite extremes of this type.

As can be seen from this brief discussion, the *Fractional Error*, which is a measure of the *confidence interval* (that is independent of scale), together with the *Signal to Noise Ratio* (which is also independent of scale), are especially convenient measures of the *confidence level*,  $P_c$ .

of the Taxonomy generated. The greater the confidence required and/or the smaller the acceptable error, the greater the processing delay and the higher the cost.

### *Circle Instructions for Writing an Improved Taxonomy Program*

1) Sample values of  $x_{ij}$  and an identification code word for each sample are entered into an active memory sequentially, and running accounts of  $n_i$ ,  $\sum_j x_{ij}$ , and  $\sum_j x_{ij}^2$  are kept to compute  $(\overline{S^2 X})_i$ . When  $(\overline{S^2 X})_i$  has increased to a value of 1,000\*, an artificial average sample consisting of the  $\bar{x}_i$ 's is recorded and the population is subdivided as follows:

2) Each entry,  $x_{ij}$ , is normalized by a factor  $k_{ij} = \bar{x}_i / x_{ij}$ , and the frequencies,  $f(k_{ij} x_{ij})$ , of occurrence of each normalized magnitude,  $k_{ij} x_{ij}$ , (in 128 uniform\* 5%\* intervals) in each row are tabulated in a 358,400\* bit memory and each normalized value is reweighted by multiplying by the factor

$$J_{ij} = \frac{-\log_2 f(k_{ij} x_{ij})}{1 \sum_i \sum_j \frac{-\log_2 f(k_{ij} x_{ij})}{\log_2 f(k_{ij} x_{ij})}},$$

$n_i$

giving  $k_{ij} J_{ij} x_{ij}$  ( $J_{ij}$  is the self information (22) for a particular entry divided by the average self-information per entry.)

3) The Tanimoto distances, i.e., the negative logarithms to the base 2 of the Similarity Coefficient, between a reweighted artificial average sample composed of attributes equal to  $J_{ij} x_{ij}$ , where  $J_{ij} = J$  for  $k_{ij} x_{ij} = \bar{x}_i$  in row  $i$ , and all other normalized and reweighted samples are computed; the frequencies of Tanimoto distances, in intervals of 0.01 units\* are computed; and a histogram is "plotted."

The "largest peak" is located in the following manner:

The modal frequency of the distribution is located. The two closest minima on each side of the mode with magnitudes *less than*  $1/8$ \* of the value of the modal peak are located (points  $A$  and  $A'$ ). The next points towards the modal peak with magnitudes *equal to*  $1/2$ \* of the value of the modal peak are located (points  $B$  and  $B'$ ). Then the next points towards the modal peak with magnitudes *equal to*  $3/4$ \* of the value of the modal peak are also located (points  $C$  and  $C'$ ). Twice\* the distance along the abscissa between points  $B$  and  $C$  (on the side nearest the origin) and between  $B'$  and  $C'$  (on the side away from the origin) is subtracted in the case of  $B-C$ , and added in the case of  $B'-C'$  to the values of the abscissa at  $B$  and  $B'$  respectively to locate new points on the abscissa (points  $D$  and  $D'$ ). The boundaries of the peak population are set by either\* the locations of the minima ( $A$  and  $A'$ ) or\* the points  $D$  and  $D'$ , whichever are *further* along the abscissa from the modal peak. (In this particular procedure,  $D$  and  $D'$  are the intersections with the abscissa of the lines drawn through points  $B$  and  $C$ , and  $B'$  and  $C'$  respectively. If the distribution function of the marginal portions of this modal peak were Gaussian, less than 4.5% of the "true" members of that group would lie beyond  $D$  and  $D'$ . If the peak were perfectly Gaussian,  $A$  and  $D$ , and  $A'$  and  $D'$  respectively would almost superimpose, and would be located quite close to the

$2\sigma$  points on the distribution curve.) The total number of samples included within the boundaries (total area under the curve between the boundaries) is recorded.

The next largest value of frequency *outside of the last defined interval* is located and the entire process is iterated  $4^*$  times. The group with the largest number of members is chosen as the "largest peak." In the event that two or more "equal" peaks satisfy these criteria, the closest\* to the origin is arbitrarily chosen as the "largest" peak.

4) A "typical" sample with Tanimoto distance from the reweighted artificial average sample equal to the distance to the peak value of the "largest" peak is selected as a candidate for Archetype of the Branch, and the Tanimoto distance between it and all other normalized and reweighted samples are computed and the procedure in 3) is reiterated until the distance between the most current candidate for Archetype and the "largest" peak drops to less than  $0.01^*$  Tanimoto units (corresponding to a Similarity Coefficient greater than 0.94).

5) The boundary of the "largest" peak is located and as many other minima (rather than  $4^*$  other — as specified in 3 above) are located as is necessary until the cumulative population included under the distribution curve out to the most currently located minimum is equal to or greater than  $95\%*$  of the population of the histogram. All samples between the origin and the boundary (as defined in 3) associated with the "most significant minimum" — i.e., that minimum such that the distance between  $D'$  on the origin side of a minimum and  $D$  on the side away from the origin, divided by the number of samples under the distribution curve between those two points is maximal — are entered into a different section of active memory as provisional members of the First Main Branch population, and the above most current candidate is elected Archetype of the First Main Branch. The Correlation Coefficient between all the normalized and reweighted provisional members of this Branch at Tanimoto distances from the normalized and reweighted Archetype greater than  $0.75^*$  units, are computed and all samples yielding values less than  $+0.10^*$  are excluded from membership in the First Main Branch. All samples at Tanimoto distances equal to or greater than  $1^*$  unit from the Archetype and less than  $1^*$  unit from the boundary as well as all excluded in the last operation are entered into a different section of an active memory as members of the First Main Residue Branch. The number of members in the Main Branch and the Residue Branch at the time of division are compared and the Branch with the largest membership is given the binary code name, 1, and the other the code name, 0. In the event that two or more "equal" minima satisfy the above criteria, the one which most nearly divides the population into equal branches 1 and 0 is chosen as the "most significant minimum."

The procedures 3), 4), and 5) are iterated for each of the above Branches (and any created by this instruction) until no further subdivision by these rules (i.e., with the same set of  $\bar{x}_r$ 's and  $j_{r\sigma}$ 's) is possible. All such branches, though constituting different levels on the dichotomous tree, define the "First Taxonomic Level."

6) Enter the next "new sample" into the active memory of the Trunk, re-computing  $(\bar{S}/N)_r$ .



a) If  $(\overline{S \cdot N})_r$  is now less than 999\*, continue to add "new samples" until  $(\overline{S \cdot N})_r$  is equal to or greater than 1,000\* and record a new artificial average sample and repeat steps 2) through 5).

b) If the  $(\overline{S \cdot N})_r$  remains equal to or greater than 1,000\* after the addition of the "new sample", remove the "oldest" sample from the active memory of the Trunk. Keep a record of  $n_T = n_r$  plus the number of samples removed since the (most recent) subdivision.

Compute the Tanimoto distance between the normalized and reweighted First Main Branch Archetype and the *new sample* also normalized and reweighted with the same set of  $\bar{x}_r$ 's and appropriate  $j_r$ 's as used to normalize and reweight the Archetype. Assign such new sample to the First Branch and/or First Residue Branch on the basis of instructions defining membership in 5), and add the new sample to the appropriate active memory or memories. Iterate with the appropriate sub Branch(es) to which such sample is assigned at the *First Taxonomic Level* in the order of descending frequency of membership in the respective Branches.

c) Each time  $n_T$  increases by an increment equal to  $n_r$ \*, compute the Tanimoto distance between the artificial average sample consisting of the  $\bar{x}_r$ 's for the current contents of the active memory and the last recorded artificial sample from 1) or 6a) (but not 6c)), and if the distance is greater than 0.01\* units, then repeat steps 2) through 6).

7) Carry running accounts of subpopulation parameters as in 1) and 6) and reiterate the instructions 1) through 7), now applied to the terminal subpopulations of a Taxonomic Level. The binary code word for the larger of the two First Sub-Branches of 1 will be 11, and the smaller, 10, and for the larger of the two First Sub-Branches of Branch 0 will be 01, and the smaller, 00, etc. Each time a renormalization and reweighting results in the *resolution* of additional sub-Branches in previously unsubdividable Branches, a new Taxonomic Level is generated. (The Taxonomic Levels may be designated, for example, by indicating the code for members of the First Taxonomic Level in upper case, the Second in lower case, the Third in upper case, etc., to provide this information by direct inspection, however, for the purposes of efficient processing of data, the added coding device is redundant and may be ignored.)

#### POPULATION STATISTICS AND THE ERGODIC ASSUMPTION

In designing the instructions for constructing the first and less efficient taxonomic classification, the assumption was made (implicitly) that consecutive samples would be picked at random from the parent population, and that the statistics of the population would be stable (i.e., that the probabilities of sampling the different kinds of patterns from the population were fixed for all time, thus defining an ergodic (10) source).

If the sampling process were not random (i.e., knowing the statistics of the source, and having collected one additional sample, we would be in a better position to predict to which Branch the next sample would belong than we were before the sample was collected), then the structure of that taxonomy, which de-



depends upon the relative frequencies of occurrence of the different patterns, might be a poor representation of the population.

The existence of both long-term evolutionary processes as well as short-term environmental changes guarantees that variations in our sampling cannot, even in principle, be random for all time and that the statistics of the population of all human serum protein patterns must change with time. We must therefore be in a position to detect changes in the sampling process and changes in the population statistics and have practical means of modifying the Taxonomy in a way that follows such changes as occur in order to maintain the usefulness of such a classification.

The  $(\overline{S \cdot N})_t$  and the intermittent checks on the similarity of artificial average samples provide sensitive indicators of changes in sampling statistics and or population statistics. With a stable source and random sampling, the  $\bar{x}_t$ 's, Branch frequencies,  $(\overline{S \cdot N})_t$ 's, and artificial average samples would all remain fixed and the updating subdivide instructions in 6a) and 6c) would never be activated. With an unstable source, only small  $(\overline{S \cdot N})_t$ 's can be used if *any short-term regularities* in the source statistics are even to be recognized.

#### INFORMATION THEORY AND TAXONOMIC PATTERN RECOGNITION

An examination of this problem from the point of view of Information Theory will be especially useful. If the population statistics are stable and each pattern is sampled independently of all others, each pattern is said to be a *discrete message*, and the parent population of samples is said to be a *discrete message source*.

The First Binary Coding Theorem of Shannon (10, 22) states that, given a discrete message source which generates information at an average rate of  $R$  bits per message, and given any  $\delta$ , it is possible to arrange sequences of binary symbols to represent sequences of messages in such a way that, on the average, *less than*  $R + \delta$  output binary symbols are required to represent the average input message from the source, but this is achieved only at the expense of a coding delay which increases as  $\delta$  approaches 0. It is not possible to find a complete representation of the source using fewer than an average of  $R$  output binary symbols per message.

If all resolved values (in our case 128) of all attributes (in this case 400) appear in the message source and all are "significant" then the message source would contain  $2^{7400}$  kinds of messages. If all possible messages occurred with *equal* frequency (an "equal likelihood source"), then the maximum value that  $R$  could take for any such message source would equal 2800 bits. If the different magnitudes of the attributes occur with *unequal* frequencies,  $R < 2,800$  bits.

If all messages are sampled at random (i.e., if the parent population is a discrete message source), and if in addition, the words of the messages (the attributes or rows) within a message are independent of one another, then the average self information per message,

$$\bar{R} = \sum_1^{m_{\text{row}}} \sum_1^{m_{\text{col}}} -f(k_{\text{col row}}) \log_2 f(k_{\text{col row}}),$$

would be equal to  $R$  for large  $n_r$ . However, the definition of "message" implies that words within a message are not independent and that the "meaning" or distinguishing characteristics of the message resides in these correlations. Therefore, while  $\bar{R}$  will be less than 2,800 bits, it will usually be greater than the real value of  $R$  because at least some of the words (attributes) of each kind of message (pattern) will show some statistical dependence on one another, and the averaging over rows in computing  $\bar{R}$  leaves out the weighting necessary to correct for this dependence.

The "mutual information,"  $I_m$  (10, 22), a measure of the complex interrelationships (joint probabilities) among occurrences of words of messages, in principle provides the required measure for correcting the value of  $\bar{R}$ . A measure of the information associated with the joint occurrence of *two* words,  $a$  and  $b$  in their respective places in a message, where the joint occurrence is designated  $a, b$  is  $I_{a,b} = -\log_2 f(a,b)$  and  $I_{a,b} = I_a + I_b - I_{a_0,b}$ . The latter equation defines the mutual information,  $I_{a_0,b}$ , between *two* words, which is equal to zero if the occurrences of the two words in their respective places in the message are uncorrelated; is positive if the joint occurrence is more frequent than predicted in the uncorrelated case; and is negative if the joint occurrence is less frequent than predicted in the uncorrelated case.

Therefore, the correct value of  $R$  would only be obtained from  $\bar{R}$  by the subtraction of the average mutual information per message (pattern) associated with the joint occurrence of all pairs, triplets, quadruplets, etc. of words in their respective positions in the population of messages. To calculate this average of the mutual information in a direct way would require very large numbers of computations and an enormous memory to store all the joint probabilities.

When some prior knowledge about the "structure" of the source leads one to believe that only the correlations between *pairs* of words (or some *other* few combinations) are likely to be significant, the task is somewhat simpler. If some simple function can be shown to permit an approximation of the average mutual information between pairs of rows of such a source, a fairly accurate measure of  $R$  may be computed without much difficulty. (Such a technique will be discussed further on.)

In examining a new information source, such prior knowledge will not be available, and the problem of both estimating the value of  $R$  and developing the "ideal code" for such a source have appeared to remain beyond present capabilities. The taxonomic approach here presented would appear to change this situation.

#### SHANNON'S FIRST BINARY CODING THEOREM, AN EFFICIENT CODE AND THE "SEMANTIC PROBLEM"

Whereas the true value of  $R$  cannot be computed directly from the raw data, the frequency of membership in a Branch Tip,  $(n_{T_i}, \sum n_{T_i})$ , multiplied by the negative logarithm to the base two of the frequency of membership in that Branch Tip, summed over all Branch Tips, converges to  $R$ , as  $n_T$  approaches infinity, if the Branch Tips are "natural" subgroups. As a result of the structure of

this kind of taxonomy and as a result of the simple instructions for coding its branches, it can be easily shown that the average length of the code words for Branch Tips (averaged on the basis of frequency of Branch Tip membership) also approaches  $R$ , the more nearly equal are the subdivisions at each Branching Point. Therefore such a taxonomic procedure is an explicit schema for the "construction" of a useful model of the two "black boxes" constituting the hypothetical "ideal" coder and decoder, the existence of which are predicted by Shannon's First Binary Coding Theorem. (These "black boxes" are also vital parts of the larger "black box" represented by a Turing machine (3).) In addition, the same sets of operations both "discover" or recognize the messages as well as "name" or code them in an efficient taxonomic code book or "dictionary." This taxonomic procedure provides unambiguous (or almost unambiguous) operational definitions which specify the *meanings* of the class membership designated by the code name. Since the semantic problem of Information Theory is "concerned with the identity, or satisfactorily close approximation, in the interpretation of meaning by the receiver, (of a message) as compared with the intended meaning of the sender" (Weaver, 10), *such a taxonomic procedure also provides a solution to this semantic problem by generating a dictionary of operational definitions.*

If our taxonomic procedure generated a symmetrical dichotomous tree with thirty levels of branching and equal numbers of samples in each terminal branch (Branch Tip), there would be a total of  $2^{30}$  Branch Tips (i.e., kinds of messages) on such a tree, and  $R$  would equal 30 bits per message. The code words generated by steps 5) and 7) would be made up of 30 binary symbols (binitis) per pattern type. This would, in fact, be a perfect Shannon-Fano Code (44, 22). It is interesting to note that whether the tree were symmetrical or unsymmetrical (i.e., different numbers of branching levels on the way up to different Branch Tips) and independently of the naturalness of the Taxonomy, this code will coincidentally always satisfy the so-called prefix condition, (i.e., no code word for a Branch Tip would constitute a prefix for any longer code word for another Branch Tip), and therefore *continuous* sequences of such code words can be decoded unambiguously. Therefore there would be no need for punctuation code symbols between messages (which necessarily lower the efficiency of a code). Thus we see that even the imperfect Shannon-Fano Code automatically generated by this taxonomic procedure has a number of the desirable properties of one of the "best possible" codes, (e.g., a Huffman Code 45, 22), which satisfy the Shannon Binary Coding Theorem. In this case, the code is generated directly, rather than retrospectively, as in the classical Huffman Coding procedure. If, after resolving the Branch Tips of such a Taxonomic Tree, we find that the variance of the information per Branch Tip is high, (i.e., the average number of symbols per Branch Tip is much larger than  $R$ ), and it is desired to provide a more efficient code, the Huffman Code for such a message source can then be generated in a straightforward manner. It should, however, be noted that while a Taxonomic Code is a significant code, a Huffman Code is a nonsignificant code (see page 446). Such a Significant Taxonomic Code has the very useful property that each prefix

of a given code word, beginning with the longest prefix and ending with the first symbol in the code word, provides the "name" of a successively more general parent population to which the Branch Tip belongs. In situations where the capacity of a "communication channel" is smaller than the rate of the information source, lopping off appropriate lengths at the ends of code words will permit us to match the source to the channel, with a corresponding but necessary loss in "semantic resolution", *but without changing "dictionaries."* By comparison, each such change in a Huffman Code requires the generation of a completely new "dictionary". A Taxonomic Code will therefore be more convenient and useful than a Huffman Code and *will require a smaller overall delay in recoding and decoding when "semantic resolution" must be sacrificed to the Procrustean bed of channel capacity.* If the measure of code efficiency includes taking the variable delays involved in recoding into account, then a Significant Taxonomic Shannon-Fano Code or similarly derived efficient Taxonomic Codes can permit us to minimize recoding delay. From this more general frame of reference (i.e., taking variable channel capacity into account) meaning is *not* "... irrelevant to the engineering problem" (10).

DECREASE IN INFORMATION AT SUBDIVISION AS A MEASURE OF  
"NATURALNESS" OF CLASSES

At each subdivision, it would be desirable to have some independent check as to whether the separation so far achieved, considering the large number of "arbitrary" boundary conditions (the \* steps in the instructions), is anywhere near optimal. That is, at one extreme, the subdivision may have no more significance than a random division of the parent population. At the other extreme, the subdivision may have separated two distinct "natural" classes of messages.

In the first case, the "true" value of each  $R$  for each sub-Branch, which we shall designate  $R_1$  and  $R_0$  respectively, would be equal to the unknown  $R$  for the parent population.

In the second case,  $R_1$  and  $R_0$  would each be smaller than  $R$  for the parent population, and  $R - ([n_{T_1}R_1/(n_{T_1} + n_{T_0})] + [n_{T_0}R_0/(n_{T_1} + n_{T_0})])$ , the difference between the information content of the parent population and the average information of the two Branch populations (which is also the average decrease in information per subdivision), would be equal to one bit if  $n_{T_1} = n_{T_0}$ , and equal to less than one bit for unequal frequency distributions.

If the subdivision is into natural subclasses, and if Branches 1 and 0 are considered as Branch Tips,  $R_1$  and  $R_0$  would each equal 0, and  $R$  would equal

$$([ -n_{T_1}/(n_{T_1} + n_{T_0}) ] \log_2 [n_{T_1}/(n_{T_1} + n_{T_0})]) \\ + ([ -n_{T_0}/(n_{T_1} + n_{T_0}) ] \log_2 [n_{T_0}/(n_{T_1} + n_{T_0})]).$$

We shall designate this measure as  $M_R$ . In this special case, the difference between the information content of the parent population and the average information of the two Branch populations, (see above), will also equal  $M_R$ .

For a given population, as different boundary conditions, (\*), are tested, this

value of the decrease in information per subdivision, calculated from the frequency of membership in the sub-Branches, can be compared to

$$\tilde{M}_R = \tilde{R} - ([n_{T_1} \tilde{R}_1 (n_{T_1} + n_{T_0})] + [n_{T_0} \tilde{R}_0 (n_{T_1} + n_{T_0})]),$$

(where the  $\tilde{R}$ 's are calculated as on page 470) and the difference,  $\tilde{M}_R - M_R$ , can be used as a figure of merit. The boundary conditions, (\*), which give the smallest difference would, in principle, be the "best" and least arbitrary. Even when  $\tilde{R} > R$ ,  $\tilde{R}_1 > R_1$ , and  $\tilde{R}_0 > R_0$ ,  $\tilde{M}_R - M_R$  should still approach 0 because most of the redundancy in the  $\tilde{R}$ 's (already discussed on page 471) is removed in computing a difference such as  $\tilde{M}_R$ . Only in so far as the statistical fluctuations (noise) in the  $R$ 's are large in comparison to the magnitude of  $\tilde{M}_R$ , will it be a poor measure. Therefore, in the limit, in the case of "natural" subdivision, as  $n_{T_1} + n_{T_0}$  becomes very large,  $\tilde{M}_R - M_R = 0$ .

Using this figure of merit to provide negative feed-back, the "efficiency" of the subdivision process can be continuously monitored, and "reasonable" boundary conditions which minimize this measure can be discovered at the very earliest stages of the construction of the Taxonomy.

This general approach is most closely related to Tanimoto's use of his entropy (Information) measure for *locating* boundaries between groups (see Rogers and Tanimoto (32), and also R. V. Smith (46)).

#### INFORMATION SPACE, THE INFORMATION TREE AND CONVERGENCE

Among the many possible spaces one may consider for the purposes of taxonomic classification, there is one which has ideal properties. We call this ideal space *Information Space*. The distance between two messages (columns),  $i$  and  $j$ , in Information Space,  $D_{i,j}$ , we call *Information Distance* ( $D_{i,j} = D_{j,i}$ ).

Suppose that a dichotomous taxonomy of "natural" classes has been generated in some unspecified way using  $D_{i,j}$  as our measure of similarity, and that the values of the  $R$ 's computed from Branch frequencies are the "true" values, (i.e.,  $\tilde{M}_R = M_R$ ). We then define  $D_{i,j}$  as follows:

$$D_{i,j} = I_{e_i} + I_{e_j} - 2T_{e_i, e_j}$$

where  $T_{e_i, e_j} = -\log_2 n_{F, e_i} n_{F, e_j} / n_T$ , and where  $F$  is the code for the first branching point backwards towards the Trunk which is common to the sub-Branches  $u1$  and  $w1$  (to which  $i$  and  $j$  respectively belong), and  $I_{e_i} = -\log_2 n_{u1} n_T$ , and  $I_{e_j} = -\log_2 n_{w1} n_T$ .

$D_{i,j}$  is therefore twice the difference between an "average self-information of a pair of messages" and  $T_{e_i, e_j}$  (which is similar to a mutual information in that it corrects this "average information" by a measure of "taxonomic correlation"). For messages for which the branching point,  $F$ , is the Main Trunk,  $T_{e_i, e_j} = -\log_2 n_T n_T = 0$ .

Consider columns  $a$  and  $b$  which are both members of "natural" Branch 1 (i.e.,  $F = 1$ ) and  $a$  is a member of "natural", "noiseless" Branch Tip 11 (i.e.,  $u1 = 11$ ) and  $b$ , of "natural", "noiseless" Branch Tip 10 (i.e.,  $w1 = 10$ ) respectively.



$$D_{a,b} = I_{ca} + I_{cb} + 2 \log_2 n_{T_1} n_T,$$

where  $n_{T_1} = n_{T_{11}} + n_{T_{10}}$  and  $I_{ca} = -\log_2 n_{T_{11}}/n_T$  and  $I_{cb} = -\log_2 n_{T_{10}}/n_T$ , therefore,

$$D_{a,b} = -[\log_2 n_{T_{11}}/(n_{T_{11}} + n_{T_{10}})] - [\log_2 n_{T_{10}}/(n_{T_{11}} + n_{T_{10}})].$$

We define distance between 1 and 11 (between a Branch and its sub-Branch) in Information Space in this case as

$$D_{a,ab} = D_{11,1} = D_{1,11} = -\log_2 n_{T_{11}}/(n_{T_{11}} + n_{T_{10}})$$

and

$$D_{b,ab} = D_{10,1} = D_{1,10} = -\log_2 n_{T_{10}}/(n_{T_{11}} + n_{T_{10}}),$$

so that

$$D_{a,b} = D_{11,10} = D_{11,1} + D_{10,1}.$$

Generalizing,  $D_{i,j}$  will be the sum of the distances from branching point to branching point, measured over the shortest path following the branches of the tree from the Branch Tip of  $i$  to that of  $j$ .

Such a binary *Information Tree* may thus be constructed "to scale" where the segments from branching point to branching point are of length  $D_{11,1}, \dots$  and width,  $n_{T_{11}}/n_T$ , etc.

Because we do not know how to compute Information Distance until after the Taxonomic Tree has been generated, we must use other distances, such as Tanimoto distance and Correlation distance between reweighted samples, as approximations to Information Distance.

From this frame of reference,  $M_R$  is the average Information Distance of a Branch from its sub-Branches, and therefore our figure of merit,  $\bar{M}_R - M_R$  may be interpreted as a measure of how closely we have approximated Information Space, and can also be shown to be a reasonable basis for establishing convergence to a "most natural" classification, (see page 456).

#### SOME SPECIAL DEVICES FOR SPECIAL PROBLEMS

The following procedure should increase the confidence that can be placed in the significance of such a figure of merit for this special case of electrophoretic patterns of serum proteins. The principle can be extended to cover sources containing considerably higher orders of complexity of joint probabilities among words, perhaps even the words in English sentences.

There are a few known properties of the information source consisting of the disc electrophoresis patterns of human serum proteins which are especially relevant at this point:

1) Protein discs have finite widths, usually of approximately Gaussian "cross-section" and greater than  $1/400$  of the total pattern; therefore adjacent rows in a pattern will be highly correlated and provide considerable redundancy.

2) In some genetic systems—for example, the case of the haptoglobin allelic

genes 1, and 2—two genotypes, 2-1, and 2-2 produce sets of different proteins. If one of a set is present, all are generally present (47), again providing added redundancy as in 1).

3) In some genetic systems, again using the case of the haptoglobin allelic genes 1 and 2, each of the three possible common genotypes, 1-1, 2-1, and 2-2, produce entirely different serum proteins; therefore if the proteins of one type are present (a particular set of rows is occupied) the others must be absent (another particular set of rows will be—to a first approximation—unoccupied) and the full set of rows occupied by the haptoglobins are therefore completely redundant, that is, given the fact that one of the set of proteins is present in a pattern, the presence or absence of all the other members of the set is determined.

4) Linked genes, in cases where the linkage has been evolutionarily stabilized (for example, by a chromosome inversion) will produce sets of proteins such that when one is present, the others will also regularly be present, and when one is absent, the others will be absent, thus providing another source of redundancy.

5) In most cases, (e.g., genes like the Transferrin gene) individual alleles result in the production of single proteins, and a heterozygous individual produces both proteins. No more than two proteins of an allelic series can normally be present in any one individual. This results in a more complex kind of redundancy which will only add appreciably to  $\bar{R}$  if more than two alleles of a gene occur with high frequency.

6) Certain pairs of proteins (e.g., hemoglobin and the haptoglobins, and a post-albumin and some pre-albumins, form complexes under conditions which depend on the concentration of these proteins in the serum. Thus, when both are present, the complex may or may not be present in addition to, or instead of, the individual proteins. The presence of such complexing phenomena also increases the redundancy of  $\bar{R}$ .

1), 2), and 3) are responsible for the major part of the redundancy from row to row. Clearly, if only their effects on  $\bar{R}$  can be reduced or eliminated,  $\bar{M}_n$  will be a very much more sensitive measure of the efficiency of subdivision.

Consider the magnitude of the Correlation Coefficient between two rows. For a pertinent pair of rows in cases 1), 2), 3), or 4), the value will be very close to 1 or -1. Since  $I_{a,b} = I_a + I_b - I_{m_{a,b}}$ , if the average self-information per word for a particular row is,

$$\bar{I}_i = \sum_i -f(k_{ri}, x_{ri}) \log_2 f(k_{ri}, x_{ri}), \text{ then } I_{m_{r_a, r_b}} \approx |C_{r_a, r_b}| (\bar{I}_{r_a} + \bar{I}_{r_b})^{1/2},$$

where  $C_{r_a, r_b}$  is the Correlation Coefficient between rows  $a$  and  $b$ , and therefore,

$$\bar{I}_{m_{r_i}} \approx \left[ \sum_{r_j} |C_{r_i, r_j}| (\bar{I}_{r_i} + \bar{I}_{r_j}) / 2 \right] \left[ 1 + \sum_{r_j} |C_{r_i, r_j}| \right]$$

and

$$R \approx \bar{R} - \sum_{r_i} \left[ \sum_{r_j} |C_{r_i, r_j}| (\bar{I}_{r_i} + \bar{I}_{r_j}) / 2 \right] / \left[ 1 + \sum_{r_j} |C_{r_i, r_j}| \right] = \bar{R}'.$$

This computation will remove the bulk of the redundancy in this kind of



message source. It also incidentally provides the means for discovering allelic series of genes of the type described in 2) and 3) and for discovering stable linkage groups as in 4). A "three-way" correlation coefficient would permit the discovery of normal allelic series such as 5) and proteins involved in complexing phenomena such as 6), and would provide a means for computing a value still closer than  $\bar{R}'$  to the true value of  $R$ . Further exploration of such correlation measures (48, 49, 50, 51) as means for computing higher orders of mutual information would seem to hold some promise for the broader application of such taxonomic programs. For clues to ways of equating, by an appropriate transformation, a measure like the Correlation Coefficient, which is an estimate of the extent of linearity of the relationship between the magnitudes of the dimensions of sets of data, to an estimate of information content like the mutual information, see Linfoot (52) and Kolmogorov (53).

#### INFORMATION THEORETICAL CONCLUSIONS

To summarize the significance of this Information Theoretical interpretation:

a) The "naturalness" and efficiency of the taxonomy generated by this technique, using any approximation to Information Distance as a measure of similarity, can be measured by means of  $\bar{M}_R - M_R$  and maximized from the very outset of the subdivision process.

b) A Taxonomy generated in a manner similar to that outlined provides a means for processing raw data in a way that results automatically both in the "discovery" and characterization of the different kinds of messages from the message source and appears to condense the information contained therein into a form approaching the limit set by theory, thereby maximizing the potential rate and accuracy with which such information can be transmitted and used. In this way, the problem of meaning, i.e., the "semantic problem," is also solved.

c) In so far as the sampling process departs from random sampling of a stable source, the frequencies which ultimately determine the structure of the binary code of the Branch Tips will change, the average information per message will change, and the proper set of normalizing "constants", the  $\bar{x}_r$ 's, will change. If the original subdivision and coding are not altered in the light of such change, the value of  $\delta$  will increase and the code will become less and less efficient. From this frame of reference, arbitrary magnitudes of loss of efficiency, beyond which loss of confidence requires that the rules for resubdivision and recoding be activated, prevent our exceeding a maximum acceptable loss in efficiency. Such confidence levels are set by instructions 6a) and 6c).

d) In so far as the source itself changes its statistics, the "naturalness" of Branches may change, requiring "pruning" of some Branches and the growth and further branching of others. The continuous updating of the Tree by addition of new samples as prescribed in the instructions provides for the necessary growth. Redivision and recoding based on updated frequencies and  $\bar{x}_r$ 's take care of pruning and also are provided for by instructions 6a) and 6c). Thus cues and mechanisms for "reeducation of the computer" are built into this scheme.

For all message sources other than absolutely stable ones, maximum effi-

ciency will only be approached in proportion to the stability (in time) of the source and sampling procedure. In the limit, for a source which is (or seems to be) continuously, rapidly and *randomly* changing its statistics, "education" is hopeless and little useful analysis of samples (messages) from such a source is possible.

#### INDUCTION, HYPOTHESIS, THEORY AND LAW OF NATURE

At this point, I would hope that the reader has developed some confidence in some of the potentials of this relatively objective pattern recognition scheme. But the development of a special purpose technique which looks at simple one-dimensional objects may seem to fall far short of my promise to deal with the "broad potentials for machine learning via automated inductive processes" since these should at least include mechanisms for discovering "causal" relationships, scientific theories and laws. In the following sections, I will try to indicate that the solutions offered by this primitive model also promise to help us to unravel most of these problems. I will try to reformulate pertinent familiar concepts within operational frames of reference which place a minimum number of constraints, derived from their more classical formulations, on their structures. Such definitions may initially appear quite unfamiliar and perhaps even in conflict with ordinary usage, but I believe sober reflection will usually result in provisional acceptance.

We will begin by asking, "What connection does the discovery of classes of protein patterns have with the generation of hypotheses?" (See 41, p. 8).

There is a set of processes closely associated with inductive inference; first, speculation, or in its more dignified form, formulation of an hypothesis; second, establishment of an hypothesis or operational definition; third, establishment of a theory, and finally, of a Law of Nature, all of which will be defined below:

a) In its most primitive and fundamental form, formulation of a simple hypothesis requires the selection of a set of objects, events or attributes from a larger population. These objects are then arranged into a class or classes, each of which is usually assumed (explicitly or implicitly) to contain other as yet unobserved members very "similar" to the members of the finite sample. As an article of faith\* in the frequency doctrine, (the metaphysical foundation of all inductive processes which has so far usually permitted useful reasoning from "the part to the whole" and from the past to the future), the probability that the sample is representative of the larger parent population is assumed to approach 1, the larger the sample and the more nearly random are any variations in the sampling technique. A description of the sampling technique itself provides the operational definition and boundaries of that part of the universe which is called the larger, "parent population," (i.e., a description of what corresponds to the "beginning" and "end" of a message), and therefore defines the message source. Were all possible boundaries in the universe observed, and were the sampling techniques completely random, then the parent population would be the class of

\* This is a necessary concession (54, 55) to David Hume's famous critique of inductive inference and is at the heart of all so-called heuristic approaches.

all classes.\*) It is then guessed that the objects belonging to such a class can be "proven" to be related to each other by objective (but ultimately imprecise) comparison of their attributes. (For conflicting points of view, see for example 8, 58, 59.)

b) Observation of the attributes of a new sample of members drawn from the larger population, together with some estimation of the confidence with which this observation appears to confirm the guess, (often in the form of what we call an experiment), constitutes a test of a simple hypothesis. (The test of a more complex hypothesis may involve drawing the new sample from a still different but "related" population.) If the magnitude of confidence exceeds some arbitrarily agreed upon level, we usually say that the hypothesis has been "confirmed." The combination of a) plus "confirmation" by b) is equivalent to the tentative establishment of an operational definition.

c) We usually dignify an hypothesis or an operational definition which concerns a very comprehensive class of objects, events or attributes by calling it a theory.

d) A Law is a theory which appears to have been established with a great degree of confidence.†

From this point of view, the generalized method—or methods, for the generation of operational definitions could include the methods for the generation of hypotheses, theories and Laws of Nature.

Let us therefore examine the operational definition more closely.

#### OPERATIONAL DEFINITIONS AND DISCOVERY

An operational definition is usually a list of the attributes common to individuals of a class which is designated by a common symbol or name. These attributes must be able to be observed or conceived by means of prescribed physical or logical operations within the limits of precision set by these operational

\* As pointed out by Wigner (56), science "aims only at the discovery of the laws of nature, that is the *regularities* of events. . . . We have ceased to expect . . . an explanation of *all events* . . ." (italics mine), and it appears that it is the "arbitrary" restriction of our field of view or attention to something less comprehensive than the class of all classes that makes it possible to learn anything at all. Our limited experience of the class of all classes is through what must be a non-random sample since the accumulation of even the very beginnings of a "representative" sample of *this* class must take an infinite amount of time.

R. J. Solomonoff (57) has also addressed himself to the general problem of prediction. He seeks solutions by searching for codes which parse a continuous string of symbols, representing the total of past experience, in a "most efficient way". Were an efficient technique developed for implementing his procedure, it might then be possible to define all the boundaries of "messages" at all levels, i.e., elementary symbols, "words," "sentences," "paragraphs," "chapters," "books" and "areas of knowledge" in an unambiguous way. His model may therefore provide a frame of reference for defining what might reasonably be meant by Wigner's "regularities of events." This problem, when looked at from the perspective of pattern recognition, turns out to be the general problem of defining "correspondence" (see page 447).

† "Laws of Nature" as used here include relationships such as the Conservation Laws as well as relationships among such relationships, e.g., the geometrical and dynamical "Invariance Principles" (56).

techniques and/or by the limits set by the logical structure of our concepts (e.g., by the Heisenberg "Uncertainty Principle" (54), Gödel's "incompleteness proof," etc. (60)). In so far as some attributes may also be shared by members of other classes, an explicit hierarchy of attributes, in order of significance, is usually part of a definition. Likewise, the hierarchical relationship of the defined class to any more comprehensive class or classes is either stated or implied in a definition.

An operational definition of a class of physical entities can be generated in two ways—each of which has a special relationship to common experience:

The first is closely related to the classical idea of the scientific method. It relies, in part, on previously accumulated knowledge of the universe. It may be identified as a "sharpening" of description or definition. It involves a class with known members which has already been informally described on the basis of subjective or vague criteria—and has been previously named. Further observation of additional members is required to determine the measure of confidence that can be placed in the definition.

The second method for generating an operational definition can be most easily identified with the process of "discovery." It requires no previous "experience" of the particular class to be defined. A "sorting" operation which discovers the class provides the information equivalent to designation of the named class and sample of known members called for in the first method, and, at the same time, may provide some explicit measure of confidence. In both cases, observation of the attributes of the members of a sample provide the "description"—in one case, usually as a hierarchical list of attributes—in the other, for example, as the maximum allowable spread in magnitude of some measure of similarity to some "typical" member of the class, (i.e., the operational specification of a decision boundary).

My reasons for focusing on the differences between these two methods of generating operational definitions is related to some apparent misunderstandings about what constitutes a valid scientific test.

For the scientific investigator, with his limited human ability to translate mental images or "visions" into words, the "subjective reality" of certain mental images inspires (rightly or wrongly) a level of confidence which is not directly and unambiguously communicable to others. He searches for a tentative communicable operational word picture or description of this image. This is his hypothesis. The external test of the hypothesis is designed to provide a basis for an unambiguous measure of confidence in this description in the minds of others, (as well as to increase his own confidence).

Because of the difficulty in communicating detailed mental images without error, it has been the classical "anti *ad hoc* argument" that "one cannot test an hypothesis with the identical set of data which was used to originally suggest it." This position can be supported as follows: In the case of a faulty hypothesis, the commission of identical errors of omission or translation is likely to instill, in the external observers, the same false confidence in the hypothesis as inspired its originator. In addition, it will usually require considerably more verbal or numerical data than is extractable from the original data set alone to provide a

communicable level of confidence comparable to the subjective one (for example, in identifying Mr. X, see page 441; i.e., "one picture is worth a thousand words").

This classical constraint might lead one to falsely conclude that the same set of data cannot be used both for discovery and for "testing" an operational definition of a class by an objective sorting procedure. But the crux of the problem of "establishing" operational definitions by either the classical or the sorting routes, is the explicit determination of an objective, unambiguous and communicable measure of confidence in the definition. When a sorting procedure simultaneously provides a completely objective measure of confidence from the same set of data that permitted the discovery of the class, then the classical taboo of the scientific method will not apply.

#### MEASURES OF CONFIDENCE, TESTS OF HYPOTHESES AND EXPERIMENTS

Most tests of hypotheses are designed to increase the level of confidence in the description of the class which is defined by the hypothesis, and the simplest "test" involves nothing more than repeated observations of the kind which provided those original data which led to the discovery of the class. Such "tests" generally result in an increase in confidence at a rate which is a function of the square root of the number of class members observed (see footnote, page 466).

An initially low communicable level of confidence in an hypothesis stems from either one or both of the following:

a) Observation has so far produced so few members that the  $(\bar{S}/\bar{N})$  is also very small and therefore the confidence in the description (e.g., the  $\bar{x}_r$ 's and decision boundary), even within a broad confidence interval, is very low.

b) Even though the number of members belonging to the class which is defined by the hypothesis is moderate, the standard deviation of the magnitudes of the attributes of the members is so large that the  $(\bar{S}/\bar{N})$  is still quite small.

If the random sampling of the parent population has resulted in only a small accumulation of samples in the particular class,  $i$ , even after a great deal of overall sampling, (i.e., very large  $n_T$ , very small  $n_i/n_T$ ), in general, to increase  $n_i$  by some factor,  $K$ , by further random sampling of the parent population will require increasing  $n_T$  by approximately the same factor  $K$ . This is clearly a slow and uneconomical procedure when the explicit goal is an increase in confidence in the particular class,  $i$ .

One resorts to the kind of test called an experiment in order to increase confidence at substantially greater rates.

One class of "experiment" consists in applying a new non-random sampling technique which ideally provides an increase in  $n_i$  at an expense no greater than that for an equal increase in  $n_T$ . Such experiments may differ from the original random sampling of the parent population by virtue of a sharp restriction in the "field of view" of the environmental transducers (measuring or sensing devices) to a more limited portion of the parent population and may be accomplished by the use of more restricted means of defining "correspondence of attributes" and/or the use of "more selective" kinds of transducers and/or the incorporation of special normalizing techniques as part of the transducer instrumentation. It



should be noted that the mechanisms of a taxonomic pattern recognition technique (normalization, reweighting, measures of similarity and techniques for defining decision boundaries) together may be interpreted as specifying the design of a highly specific, ordered set of "sorters," "sieves" or "filters" for efficiently separating the members of many classes from the mixture that is the parent population. A well designed experiment may therefore be interpreted as involving taking the relationships already discovered among the attributes of the observed members of the class and "related" classes as well as the statistics of such classes as bases for the design or "invention" of a *special purpose filter*. With such a filter, one hopes to efficiently sieve new members of the particular class from a portion of the parent population—ideally in a single "sweep."

Another kind of experiment can involve an attempt to observe the parent population or its members at a higher level of resolution, and/or over an expanded range of magnitudes of the already known attributes, and/or with the examination of "new" additional attributes not included in the original observations of the parent population, and/or with new normalizing techniques, new similarity measures and/or new decision boundary criteria.

The first class of experiments is mainly aimed at efficiently acquiring a large increase in  $n_i$ . In so far as large standard deviations (in case *b*) stem from "errors in measurement" rather than "true" variations in the attributes of the members of class *i*, such an experiment may secondarily also lead rapidly to a high  $(S/N)_i$ , and a high level of confidence if the special purpose filter introduces smaller errors of measurements.

The second class of experiments is aimed directly at reducing the standard deviation by either reducing the weight of variation among the magnitudes of the original set of attributes in the hope that the new set is both characteristic of the class *i* and relatively homogeneous, and/or by reducing the weight of errors of measurement, and/or by reducing the magnitude of the errors of measurement.

I believe that analysis of all kinds of scientific experiments (with the exception of one remaining class to be discussed a little further on) can be reduced to either one or a combination of the two classes just discussed. Such analyses suggest that experiments can, in principle, be performed by "aiming" a special set of environmental transducers ("sense organs") at a restricted subset of the class of all classes and subjecting their outputs to analyses by a special purpose taxonomic pattern recognition program, designed on the basis of past experience with the particular subset and "related" classes. Experimental routines are therefore reasonably compatible with the operations of a general purpose taxonomic pattern recognition machine.

(We have in fact proposed to test the diffuse hypothesis, "The set of soluble, circulating, direct transcriptions of the genetic code that are the serum proteins, will vary in kind and concentration as a function of the physiological states of the human organism," with an experiment that consists in "aiming" our pattern recognition program at a very restricted subset of the class of all classes.)

The economy gained by this process of narrowing our field of view is bought at the price of the introduction into the inductive scheme of a system of values

related to specific intentions, goals or "purpose" (and elevates us to the Third level of Information Theory (10)). To choose a particular class that we consider warrants the special attention of an experiment from among the large number of classes which have already been "discovered" (by, for example, an "unbiased" pattern recognition device as it selects samples "at random" from that part of the universe most proximal to it), implies that this class has higher intrinsic value (for example, higher adaptive value) than those other classes which have failed to receive such "privileged" treatment.

Therefore, to introduce "experiment" as an automatic part of a general purpose inductive taxonomic program will require providing the machine with an "acceptable" set of operationally defined "goals" or "values." This can provide a general purpose taxonomic pattern recognition machine with the flexibility to "specialize" and considerably increases its versatility and utility at a relatively early stage of its "education." But it must be kept foremost in our minds that it is just at this point that the possibility of the choice of "an unfortunate" set of values could pose an even more serious threat to mankind than, for example, the choice of anti-social values as the guiding principles for a "mere" human.

#### CONFIDENCE IN HIGHLY "STRUCTURED" THEORIES

One important aspect of the scientific method which has so far been intentionally neglected in this treatment concerns what might be considered to be still another kind of scientific hypothesis or theory. Such an hypothesis represents something very close to a mathematical theorem, the "form" of which has been perceived by the investigator and has been expressed in verbal or symbolic form. The "test" of such an hypothesis often depends more upon the logical manipulation of relations in which we may, from past experience, already have a great deal of confidence, (as axioms and fundamental propositions are manipulated logically in the proof of a theorem), than on a new observational test. This "mathematical" kind of hypothesis building and testing is more akin to chess playing and language manipulation. It constitutes a very important element in science and is the area where the application of the computer has so far been most promising (41, 61). To summarize, the building of confidence in such theories or hypotheses properly relies on logical deduction from "established fact" perhaps even more than on new empirical induction. By contrast, beautifully consistent, logical castles built on patently weak foundations (i.e., we have low confidence in the premises) demand independent direct verification before they are even likely to be given any attention by a knowledgeable scientific community. Such theoretical construction plays a major role in the so-called method of strong inference (58, 62). This explains the third function of experiment in the advanced sciences. Such an experiment is designed to provide a new kind of observation which will provide an explicit objective measure of confidence to substitute for and or supplement logical "confirmation." (When the premises have been only weakly established, a successful experiment can also provide increased confidence in the "axiomatic elements".)



Because weakly supported hypotheses are by far the more numerous in our experience, many scientists do not easily recognize that there is, in fact, a distinction to be made and they are therefore regularly more "comfortable" with those hypotheses which are directly supported with empirical as opposed to logical tests or "confirmation." This is better understood when we remember that "logical confirmation", so far, usually depends upon a less explicit and therefore more ambiguous measure of confidence acquired by mental manipulation of the joint confidence in sets of earlier observations (e.g., those confirming Laws of Nature). To illustrate my point: 1) Although Einstein replaced a growing mountain of *ad hoc* and often contradictory hypotheses (63) with a comparatively simple and much more comprehensive theoretical structure, many initially had little confidence in his Theory of Relativity because it depended upon "deductions from" fundamental invariance principles which were derived, as a set of "axioms," from the already empirically established high confidence in Newtonian mechanics, Maxwellian electrodynamics, the measured "constancy" of the velocity of light in "vacuum" (independent of the velocity of the frame of reference relative to other frames of reference), and the measured "equivalence" of inertial and gravitational mass. 2) Since "confirmation" with results from a computer is not yet available, many will have little confidence in a substantial part of what appears in this manuscript because any attempt to derive a comfortable measure of confidence by mental manipulations must be non-numerical and vague. This is necessarily true because some of the component elements must be derived from a) the confidence in the past successes of Information Theory, b) confidence in those more remote empirical bases in experience with the technology of communication and with those analyses of language from which Information Theory has been derived, and c) from the non-numerical personal confidence of the individual readers in, for example, the relevance of his own observations of the way he and others classify and process data to the infant-taxonomist analogy of my introduction.

Yet, in contrast, the explicit numerical levels of confidence which can be recorded for those class descriptions which may be provided by a taxonomic computer program are, in principle, amenable to quantitative manipulation to produce net measures of confidence in the combined observations. For example, were all words of all messages independent of one another, it can be easily shown that the weighted root-mean-square value of the Signal to Noise Ratio for words (rows),  $(\bar{S}N)_r$ , (see page 466), provides a reasonable quantitative basis for ascribing an unambiguous measure of joint confidence in the overall mean value of the  $\bar{x}_{r_i}$ 's.

Since words of messages are usually correlated, the weight of the individual values of the  $(\bar{S}N)_r$ 's must be reduced by some measure of the average mutual information per word (as on page 476), in order to correct for any redundancy which would otherwise lead to falsely high values of  $(\bar{S}N)_r$ . It can be shown that a more accurate value of  $(\bar{S}N)_r$  which takes such redundancy into account is,

$$(\overline{S/N})_r \approx \left[ \sum_{r_i} \left( 1 - \frac{\sum_{r_j} |C_{r_i, r_j}|}{1 + \sum_{r_j} |C_{r_i, r_j}|} \right) \right] \cdot \left[ \sum_{r_i} \left( 1 - \frac{\sum_{r_j} |C_{r_i, r_j}|}{1 + \sum_{r_j} |C_{r_i, r_j}|} \right) (N/S)_{r_i}^2 \right]^{-1/2}.$$

Such kinds of manipulations can provide the required explicit measure of joint confidence in multiple observations with varying individual levels of confidence while at the same time correcting for redundancy (64). Such measures could substitute for the at present more ambiguous measures of confidence which must be used to judge those theories which are operational definitions of comprehensive classes (of still more primitive but well characterized classes) that have not yet been independently tested by appropriate new observations (by experiments). Indeed, such derived measures will often provide sufficient confidence to permit the useful by-passing of much (sometimes all) repeated independent testing by new kinds of experiments. This has often been the case with careful and knowledgeable engineering design from principles for which past experience has provided a great deal of confidence. Similarly, some physical theories, such as the Theory of Relativity, are grounded on an even more extensive, comprehensive and therefore more convincing, observational base and have, as a result, required relatively few new observations to establish them quite firmly.

This position contrasts with a rather widely and I believe wrongly held view that, "The idea of determining the numerical value of the probability of scientific theories seems preposterous" (65), because as I have suggested above, such measures of confidence could be provided both from direct relevant observation and/or indirectly through estimation of the joint measure of confidence in those pertinent, "more primitive" observations which are to serve as a base for those operational definitions which function as the fundamental propositions of a "mathematical", scientific theory.

My efforts on these pages have been largely directed at shoring up confidence in the promising possibilities of machine approaches to inductive processes, albeit, mainly by the use of what, though sketchy, I hope will be found to be logically consistent arguments deduced from observationally rooted premises. In the process, I hope I have incidentally helped to destroy some of the remaining apparent magic in science, and have perhaps provided another strand of understanding for a bridge between the "Two Cultures" (66, 67).

#### PATTERNS OF HIGHER DIMENSIONALITY

Given the raw data equivalent to the experience which has been the source of previously accumulated knowledge, an appropriate sorting method for generating operational definitions will, in principle, generate definitions for all the classes which have been generated by the classical scientific method—but with a much

more comprehensive and clean-cut taxonomy. To repeat all analyses of the past would be extremely time consuming, but when faster computers become available, the devotion of a number of years to the "education" of a general purpose computer may become reasonable. An appropriate sorting method for generating operational definitions will probably be found to be closely related to the newborn's learning process; is directly related to Dr. Tanimoto's "Elementary Mathematical Theory of Classification and Prediction" (31); and I believe is, in fact, something very close to the procedure which I have outlined for the discovery of protein pattern classes. The definitions generated by such a procedure become formally equivalent to classical simple definitions if one uses a measure of similarity between rows of attributes for each class (using the normalization and reweighting appropriate to the next most general Branch population from which it was derived) to generate each appropriate descriptive hierarchical list of attributes.

Each serum sample provides us with a one-dimensional pattern, equivalent, for example, to an electrocardiogram, to an absorption spectrum, to a single line in the raster of a two-dimensional television image, etc. In so far as the techniques outlined are of a fundamental nature, the same approaches should be fruitful in the recognition of two-dimensional images—for example, pictorial representations such as microscopic images of cells (in which I am particularly interested), alpha-numeric symbols and words in printed and written texts, phonograms, etc.

The problems involved in going from 1 to 2 dimensional patterns raise formidable, but I believe solvable, topological complications, (68, 69). If the solution of these is achieved at a level which keeps the problem of correspondence tractable (e.g., see 70), recognition of  $n$  dimensional patterns looks extremely hopeful.

The visual and auditory pattern recognition apparatus possessed by most mammals appears to permit a kind of recognition and discrimination of, for example, face and voice that closely matches human capabilities. For example, most intelligent dogs can recognize a large number of "friends" by sight or sound (as well as "smell"). The evolution of this complex apparatus (sense organs and neural structures) occurred over a period of more than 100 million years. It appears that the small amount of mutational innovation and evolutionary remodeling that can occur in about a million additional years was sufficient to take man across a new data processing threshold. This gave him his meager ability to translate his "high resolution" mental images into sets of symbols for social communication that are somewhat more sophisticated than those of other mammals. Yet despite its very primitive stage of development, this ability to translate (page 422) has apparently taken him most of the additional distance he has come.

For some people, "thinking" mainly involves mental verbalization of both a problem and its detailed solution, but for at least a few of our most creative thinkers (e.g., see 71), pages 83-99 and Einstein's letter in Appendix II), most of their thinking apparently involves mental manipulation of non-verbal patterns. One wonders whether the common admonition to "think things out verbally," by perhaps confusing the translation problem with the "analytical" problem, may not have extensively handicapped the formally educated population of the world by largely restricting the bounds of human imagination to the

inefficient and low resolution domain of digital symbol manipulation which follows the "translation step." We have the very common handicap to rapid and comprehensive reading (i.e., at 1,000 to 2,000 words per minute as compared to 200 to 400 words per minute, see for example (72, 15)), that appears to stem from such poor reading habits as resorting to "sub-vocal" "inner speech" (i.e., "saying words of the text to oneself, one by one") as a striking and possibly analogous case.

Intuition derived from a background of "biological experience" suggests that once a general solution to the pattern recognition problem has been developed, a few comparatively simple innovations in the deductive techniques for the machine translation of natural languages (73, 74, 75, 76) should permit us to rapidly boot-strap the computer far past man, (however see some of the reservations of both Bar-Hillel (77) and Selfridge (9)). Since the structure of the language of a pattern recognition program for a digital computer will be explicit and known, in contrast to the case with the "language" of the gestalt (and in contrast to the case with the "language" of a perceptron (78, 79, 7)), we anticipate no problems related to a "translation barrier." Therefore, even without deductive innovations, the range of application of a satisfactory pattern recognition program may exceed the capacities of most mammals and perhaps even man.

#### TIME, CAUSALITY AND A MECHANICAL ORACLE OF DELPHI

Except for the inferences that may have been drawn from the occasional suggestion that events might be treated as patterns, such taxonomic procedures may seem irrelevant to the discovery or generation of "causal" hypotheses, theories and laws. However, if an event is observed as (or can be transformed so as to correspond to) an ordering of attributes along a time axis, then when events are compared to one another, those which are found to be very similar will often obey the same "causal laws." The occurrence of high correlations or similarity between the attributes of such events provides information equivalent to the usual kinds of causal statements associated with processes, i.e., the earlier attributes are either the "causes" of the later attributes and or both earlier and later attributes result from "common causes," (e.g., the "Law" which is the operational definition of the class).

In particular, if useful transformations from the relativistic four-dimensional time-space continuum to one dimension can be developed, then all aspects of causation will, in principle, be amenable to such taxonomic pattern analysis. In the meantime, many relatively simple "one-dimensional" processes or events (e.g., electrocardiograms) will be analyzable by even this primitive kind of model.

Since it has been convincingly argued that  $n$ -dimensional pattern recognition is analogous, if not homologous to what we generally call thinking (3, 4, 5), we may be close to having the blueprints for a mechanical Oracle of Delphi.

#### CONCLUSION

It would appear that a primitive frame-work for a process which fulfills most of the functions of the "scientific method" can probably be programmed for digital computers. It provides the mechanism for both "discovery" and for auto-

matic generation of operational definitions. From these, the symbolism which is the grist of the "more sophisticated part" of the scientific mill can be derived.

Is the more sophisticated aspect of the scientific method, the generation of hypothetical models followed by testing these models by further select observation, really different in kind from the more primitive process?

I have already indicated my belief that it is not. The level of complexity of the attributes of "objects" to be compared may be greater, but the added sophistication comes mainly from the use of additional and more complex "measures of similarity" and/or more complex forms of normalization. For example, the Correlation Coefficient permits us to discover the existence of any linear relationships between two sets of data,  $x$  and  $y$ , such that  $y_i = mx_i + b$  for all values of  $m$  and  $b$  except  $m = 0$ . If one performs the non-linear transformation represented by taking the logarithms of the data points, then the Correlation Coefficient, when applied to such transformed (normalized) data will detect any relationship between the two sets of data such that  $y_i = cx_i^n$  for all values of  $n$  and  $c$  except  $n$  or  $c$  equal to zero.

If one considers equations to which data are usually fit in "scientific models" (e.g., linear and non-linear algebraic and differential equations, etc.), semi-metric measures and transformations probably exist which have a similar relationship to these equations as the Correlation Coefficient in combination with a logarithmic transformation has to simple exponential equations. If such additional similarity measures and transformations were added to this type of pattern recognition program, much of the more elegant aspects of the scientific method might, in principle, be relegated to the computer (especially if some sophisticated heuristic can be developed for determining which data points in one set "correspond" to the data points in another).

Golomb (80) has stated that "it is scarcely an exaggeration to assert that *classification* is the most fundamental objective in mathematics," (italics mine). It would therefore seem reasonable to expect that efforts of socially motivated mathematicians might be easily and profitably turned in these directions. Many of the voids to be filled seem to be directly related to Topology, (particularly Algebraic Topology), Set Theory, Group Theory and the very broad areas of Analysis.

Let us suppose that the ideal set of analytical techniques had already been produced by the mathematicians, and that we were convinced that such techniques should be applied to the analysis of the widest possible range of problems. What remaining bottlenecks can we envisage?

Present day computer technology has (for good reason) been mainly committed to the development of computers that handle most data in their arithmetic sections sequentially. If an analytical scheme requires the computation of 10,000 similarity coefficients, the time necessary to perform such computations must be approximately 10,000 times the time to compute one similarity coefficient because the same hardware must be used sequentially for each of the individual computations. With the advent of "integrated micrologic circuitry" which occupies much less volume, consumes much less power and is faster and potentially



more reliable than existing circuitry (81, 82), industry is beginning to plan for machines which have considerable parallel capabilities (83). However, only one serious effort seems to be directed towards the ultimate construction of a machine with anything approaching 10,000 parallel arithmetic sections (84). Yet because of the enormous numbers of computations required for such an analytical taxonomic scheme, such machine capabilities plus a high-speed memory 100 or more times the capacity of the IBM 7090, would probably be mandatory to make it economically feasible to use a taxonomic program for the solution of the widest range of problems. It would appear that the remarkable capabilities of the vertebrate brain (and especially the human brain), in spite of the comparatively slow speed of the individual "circuits," are largely attributable to the enormous numbers of "parallel circuits" at all levels of the nervous system, (e.g., see 85, 86, 87, 88). Given such a quantitative change in computer design, we should expect as striking an advance in data processing capabilities as in the evolutionary transition from protochordate to man. Since, in contrast to the mutational part of the evolutionary process, the technological innovation would be goal oriented, this kind of data processing can change man's way of life at a staggering rate.

Let us suppose that the social and industrial motivation were sufficient to propel us rapidly along the road to widespread use of analytical taxonomic programs on large-scale, parallel-circuit, digital computers for the solution of the widest range of human problems. Will we humans be prepared to cope with the intellectual, cultural and emotional problems that are likely to be created by the most severe and extensive case of technological unemployment we have ever had to face? (89) In competition with the machine, most, if not all of us, may be found to be amateurs even at that scientific game of chess that we play with nature—and which we call research (6, 9, 90). Many of the problems posed by this kind of question may demand solutions within the next few generations. Viewed from such a perspective, from among the most knowledgeable estimates of the formal educational needs of our younger generations, (e.g., 91, 92), none comes even remotely close to the mark. In the words of the late Norbert Wiener, father of Cybernetics, who was troubled by similar considerations, "The hour is very late, and the choice of good and evil knocks at our door" (93).

#### SUMMARY

The purpose of this paper has been to examine some aspects of the general problem of learning in terms of pattern recognition.

In our approach, we start with a detailed "image" of each pattern of the set, placing a minimum number of arbitrary constraints on the limits of the raw data, (e.g., setting only a maximum level of resolution and the outer bounds of each image field). The data points of the image or pattern are then represented as the coordinates of each pattern, and the pattern itself, as a point in an  $n$ -dimensional space (hyper-space), and a semi-metric measure of "similarity" of pattern to pattern is used to define a distance between samples and characterizes this hyper-space as a semi-metric space (rather than a metric space). The semi-metric distances between patterns are examined and the largest "natural" constellations

or clusters are separated from one another. The data points of patterns within each constellation are reweighted, based on the information content of the subpopulation, and are further divided into subgroups by the same techniques. Those boundary conditions which must be arbitrarily set at the outset can be systematically varied to produce the maximum decrease in information at each subdivision. This serves as the device for converging to the "most natural" subdivision by measuring how closely our semi-metric space approximates a defined, ideal "Information Space", thus removing a large element of remaining "arbitrariness" from the procedure. In this way, a taxonomy of patterns is automatically generated. By introducing a simple binary coding device which assigns 0's and 1's to designate the smaller and larger constellations at each subdivision, an efficient Shannon-Fano Code (10, 22) (in terms of Shannon's Binary Coding Theorem (10)) for the "description," "communication" and processing of such patterns is automatically generated. Such a taxonomic procedure both "discovers" classes of patterns and orders them in a "dictionary" and provides the means of efficiently recognizing whether any new sample of a pattern "belongs" to one or more of these already discovered classes. It thus solves the "semantic problem" of Information Theory (10). Mechanisms for continuously updating the process on the basis of the statistics of the portion of the population of patterns already sampled and analyzed are explicitly introduced into the scheme.

This technique departs widely from the more familiar decision-space model of decision theory, (e.g., see 30, 94, 95, 96, 97, 98, 99, 100), where, at the outset, specific "teleological" constraints are usually placed on a limited synthetic set of pattern parameters which it is hoped will allow the differentiation and recognition of the different kinds of known patterns; where a metric decision-space is usually carved up by sets of "hyper-planes" which are intuitively and computationally more complex and usually more arbitrarily distributed than our decision boundaries; and where no attempt is usually made to structure the resulting set of classes in an efficient "dictionary" or taxonomy.

The relationship of our model to "learning" and to the "scientific method" is discussed. The areas of mathematics which might be further exploited to increase the sophistication and generality of such procedures are noted. The pertinence of computer design to the efficient and widespread application of such a class of techniques is briefly discussed and a change in direction is recommended in this connection. Attention is finally drawn to potential social consequences of the widespread use of such techniques.

#### ACKNOWLEDGMENTS

This work is dedicated to the memory of Mount Sinai's Paul Klemperer. As a result of my close and continuous association with Dr. Klemperer from 1955 until his death in 1964, this great pathologist's continuing concern with the explication of the historical foundations of modern concepts of medicine and science (e.g., see 101) provided inspiration for a good portion of this inquiry.

It was also through the stimulus provided by frequent discussion with T. T. Tanamoto of the basic ideas in his "Elementary Mathematical Theory of Classi-



fication and Prediction" (31) over the period from 1957 to 1961 that most of the viewpoints presented here first began to take shape. Both Dr. Tanimoto and my colleagues, B. J. Davis and S. Diamond have persevered as constant sounding boards through many changes in direction, and have provided most of the necessary negative feedback which has led to this fairly stable and, hopefully, useful perspective.

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# Male Urogenital Trichomoniasis

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## INTRODUCTION

Since the discovery in 1836 by Donne (1) of *Trichomonas vaginalis* in the vaginal secretion of women suffering from leucorrhea an extensive literature has accumulated describing the infestation caused by this flagellate. Most of the papers and research work however has been directed toward eradication of the protozoa from the female genito-urinary tract. Little attention has been focused on the problem of *Trichomonas vaginalis* in the male.

*Trichomonas vaginalis* infestation of the male genito-urinary system is more frequently encountered than was formerly suspected. It took a long time to come to the agreement of today that this stubborn organism—*Trichomonas vaginalis*—cannot be eradicated if the male partner is not investigated and treated. Only through close co-operation between the urologist and gynecologist will a complete cure be assured.

## REVIEW OF THE LITERATURE

The first two reported cases of *Trichomonas vaginalis* infestation in the male appeared in 1894 in the *Zentralblatt für Bakteriologie und Parasitenkunde* by F. Marchand (2) and K. Miura (3). F. Marchand (2) reported a case of a 60 year old male who had suffered from a perineal fistula for seventeen years. In the absence of any symptoms of cystitis the patient developed turbid urine which contained a yellow sediment and numerous whitish particles.

Microscopic examination of the sediment showed in addition to numerous bacilli, cocci, epithelial cells, hyaline casts and erythrocytes, the presence of flagellates. Comparing these flagellates with the ones found in the vagina of certain females which he knew were *Trichomonas vaginalis*, Marchand (2) concluded that there was at least a resemblance if not identity between them.

In the same year K. Miura (3) (1894) of Tokyo described the case of a 52 year old Japanese male who complained of left flank pain. The freshly voided urine, about 200 cc, was yellow in appearance with an acid pH and did not contain albumin or sugar but many 2-5 mm long shreds and whitish floccular forms, similar to the "short shreds" of gonorrhea. The microscopic analysis disclosed many round cells, epithelial cells and motile infusoria which were larger than the pus cells and identified by Miura as *Trichomonas vaginalis*. Miura described the flagellates in detail but mentioned the presence of one flagella, rarely two or three. Marchand (2) who was the official re-

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viewer of Miura's paper disagreed with this finding and attributed it to the lower-power microscope used by Miura. Nevertheless, Miura's paper was very accurate in all other details. The author used the two glass urine test and left an indwelling catheter in the bladder in order to determine the exact site of infestation: kidney, ureters, bladder or urethra. Only the first glass contained the shreds while the urine obtained through the catheter was free of protozoa. Therefore, Miura concluded that the site of infestation is the urethra. It is also Miura's merit to have established the source of infestation. In order to determine this Miura examined the vaginal secretion of the patient's wife and found that it contained *Trichomonas vaginalis*. Miura concluded that infestation was transmitted during coitus.

Dock (4) (1896) presented the case of a 27 year old man who suffered from painful and difficult micturition. The urine contained large flakes consisting of pus and epithelial cells and numerous flagellates.

Katsumuma (5) in 1924 reported the case of a 3 year old boy with severe balanitis caused by *Trichomonas vaginalis*. The preputial sac harboured the flagellates.

In 1925 Dastidar (6) reported four cases in which he recovered the protozoa by centrifugating the urine during routine examination of some 1000 specimens of urine derived from both sexes. Three patients were males and one was a female. He concluded "that *Trichomonas vaginalis* may cause a mild urethritis attended by smarting or burning on micturition or even a discharge from the urethra, but this urethritis cures itself with the disappearance of the protozoa."

Capek (7) reported two interesting cases of *Trichomonas vaginalis* urethritis in the male in 1927. The urethritis manifests itself in the male by a purulent urethral discharge, many non-specific organisms, numerous trichomonads, many red blood cells and large amounts of pus and fibrin. One patient was married and, on examination of the wife, protozoa were recovered from the vagina, cervix and urethra.

Riba and Perry (8) in 1929 described two cases of *Trichomonas vaginalis* urethritis in the male complicated by *Trichomonas prostatovesiculitis*. The expressed prostatic fluid revealed active motile trichomonads, the urine contained many shreds and motile flagellates. Riba (9) (1931) states that among 3000 specimens of prostatic fluid examined nine cases of prostatovesiculitis associated with *Trichomonas vaginalis* were found giving an incidence of 0.03%. Riba believed this percentage was too low. He emphasized the importance of examining the fresh urethral and prostatic fluid in order to diagnose *Trichomonas vaginalis* in the secretions. The motile protozoa were readily demonstrated in fresh wet preparations.

Grimm (10) (1930) reported five cases of *Trichomonas vaginalis* infestation in males. Four patients were single. The fifth patient was married and his wife suffered from *Trichomonas vaginalis*.

Rosenthal (11) (1931) reported a case of urinary infection with *Trichomonas vaginalis* in a 72 year old male manifested by an acute attack of



pyelitis. The urine in addition to pus cells and coliform organisms contained *Trichomonas vaginalis*.

Stuhler (12) (1933) commented on the results of the examinations on prostatic secretions. Of 32,000 specimens of prostatic fluid examined at the Mayo Clinic, *Trichomonas vaginalis* was found sixteen times. The technique of the examination was not described and the accuracy of the series is open to question. There is a marked difference between Stuhler's (12) and Riba's (9) findings. In Riba's (9) series the incidence was 0.03% which the author believed was too low because many of the microscopic examinations were carried out by medical students not sufficiently experienced in the art of microscopy. Subsequently many other observers reported not only urethritis but also cases of prostatitis and epididymitis caused by *Trichomonas vaginalis*. The clinical entity of male trichomoniasis was definitely established and accepted. The importance of the husband as a potential source of reinfection was soon recognized.

Allen, Jensen and Wood (13) (1935) reported six cases of *Trichomonas vaginalis* in husbands whose wives suffered from *Trichomonas vaginalis*. The flagellates were found in fresh films of prostatic fluids.

Cornell and Riba (14) (1936) reported that in seven years they encountered thirty cases of *Trichomonas vaginalis* infection in males. Of the 30 men, 25 were married and the author expressed the belief that *Trichomonas vaginalis* in the male is acquired solely through sexual contact.

Drummond (15) (1936) in a similar study investigated the five husbands of women suffering from *Trichomonas vaginalis*. He examined the fresh prostatic fluid and cultured the secretions and was able to demonstrate the presence of *Trichomonas vaginalis* in the prostatic fluid in four out of five cases. Interestingly enough none of these patients presented any symptoms.

Nitschke (16) (1936) found five cases of *Trichomonas vaginalis* infection among forty cases of non-specific urethritis. In all these cases the female partners of the infected men suffered from *Trichomonas vaginalis*.

Karnaky (17) (1938) investigated the cause of recurrence of *Trichomonas vaginalis* vaginitis and found the protozoan in 38 of 150 husbands of infected women. The flagellates were found in the preputial sac, urethra and prostate.

Liston and Lees (18) (1940) investigated 400 men attending a venereal diseases clinic and found that 16 patients (4%) harboured *Trichomonas vaginalis*. Only one of the 16 patients had gonorrhea while the others had been classified as having "non-specific urethritis."

Kolesoff (19) (1950) described the action of *Trichomonas vaginalis* on spermatozoa *in vitro* stating that the spermatozoa first lost their motility and then were ingested by the protozoa. The ones who escaped this fate were lysed within three hours. These observations have not been confirmed by others.

Whittington (20) (1951) studied sub-fertility patients, having thus the opportunity to study both partners and examining concurrently the husband's semen and the wife's vaginal discharge. The seminal fluid (semen) was pro-



duced by masturbation or coitus interruptus. The author examined seminal specimens from the husbands of 26 women who were or who had been recently infected with the parasite. The semen was examined by using the fresh material microscopically as well as by culturing it. Seven (27%) of the men were found to harbour the flagellates but only one had subjective symptoms. In a control group comprising 10 husbands of *Trichomonas vaginalis* free women, no flagellates could be demonstrated in the semen. The author emphasizes the intimate connection between the infection of the husband and the wife and the great importance of treating both partners simultaneously. The infection is acquired many times in coitus. Clinical evidence, cited by Drummond (15) (1936) and Pattyson (21) (1937), supports the view that *Trichomonas vaginalis* is transmitted venereally; however the failure to find the flagellates in three-fourths of the husbands of infected women is in contrast to the conditions generally established and accepted for the transmission of venereal diseases such as lues or gonorrhea. Therefore a definite distinction has to be made between *Trichomonas vaginalis* and venereal diseases as ordinarily understood.

Lanceley and McEntegart (22) (1953) inoculated intra-urethrally five volunteers with a pure culture of *Trichomonas vaginalis*. Three of the patients who received the protozoa culture developed urethritis from which the protozoa were recovered for different periods of time. The remaining two patients developed a mild transient urethritis but no trichomonads were recovered at any time. Serum was examined by the hemagglutination method for the presence of antibodies of *Trichomonas vaginalis*. None of the samples gave a significant titre of agglutination thus failing to demonstrate antibodies to the protozoa.

#### INCIDENCE

Fco, Varano and Fetter (23) (1956) reviewed the incidence of *Trichomonas vaginalis* in the urethritis of the male. They state the incidence of non-specific urethritis among urethritides varies from 12.4% to 70% according to various authors and sources [Bull. U. S. Army Med. Dept. 1947 (24); Crouch, Reese and Boudreau 1953 (25); Parrino 1954 (26); Babione and Graham 1952 (27); Durrel and Siboulet 1954 (28); Harkness 1950 (29)]. The high incidence of *Trichomonas vaginalis* in the male population and in males with non-specific urethritis has been an established fact [Bauer 1942 (30); Fco 1944 (31); Seneca and Ides 1953 (32); Leca 1951 (33); Kozlowski 1951 (34); Sorel 1952 (35); Lanceley 1953 (36); Jira, Rossler and Svejcar 1955 (37)].

Fco *et al.* emphasized the role of *Trichomonas vaginalis* as an etiologic agent in cases of non-gonococcal urethritis. In a series of 183 male patients with urethral discharge, 108 (59%) were found to have gonorrhea. The remaining 75 patients had non-gonococcal urethritis, of these 33 patients harboured *Trichomonas vaginalis*; this incidence represents 18% of all men studied and 41% of the non-specific cases.

The incidence of male trichomoniasis in our series of 94 patients is 28% (see Table I).

#### CLINICAL COURSE OF TRICHOMONAS VAGINALIS INFESTATION IN THE MALE

In the male *Trichomonas vaginalis* is found in the prostatic and seminal vesicle secretions, urethral discharge and urine of infected individuals. Its habitat in the male is in the urethra, where it may invade the epithelium, and in the prostate. These parasites infestate the male urethra and prostate gland at times without symptoms and at other times provoking severe inflammatory reactions.

In many cases *Trichomonas vaginalis* infection in the male may be latent (*i.e.*, symptomless) at the time of physical examination but later be responsible for a stubborn and tenacious urethritis which does not respond to the routine therapy.

Material to be examined microscopically consists of the urine and secretions obtained from the prepuce, urethra, prostate and seminal vesicles. In order to obtain prostatic fluid, the gland is massaged while seminal vesicles secretions are obtained by masturbation.

Since *Trichomonas vaginalis* infestation of the male appears to be very often in the latent stage, the infection is more likely to be suspected and diagnosed in gynecology rather than urology clinics (39). This is particularly true in the case of male patients suffering from non-specific urethritis in whom casual and superficial examination may lead to erroneous diagnosis and the prescription of a drug which is not trichomonacidal. When one member of the pair is found to be infected, he or she must be convinced that it is essential to postpone sexual contact until the infection has been eradicated. Laboratory studies should accompany clinical examination to confirm or to rule out trichomoniasis. Only by following these criteria can infection be prevented or controlled within the mated pairs. It is also incumbent upon physicians when examining male patients to supplement physical observations routinely with protozoa laboratory tests to determine if the patient is an active or latent carrier of *Trichomonas vaginalis*.

The first clinical manifestation of *Trichomonas vaginalis* urethritis may become evident within a period of twenty-four hours or several days following sexual relation with an infected partner. The severity of symptoms varies considerably and depends on the area infected by *Trichomonas vaginalis* (18). The stages of the disease may be graded according to the anatomical area infected by *Trichomonas vaginalis*.

a. Infestation of the Prepuce: This area offers an ideal culture ground for the growth of *Trichomonas vaginalis*. Proper hygiene and circumcision are good preventive measures.

b. Infestation of the Urethra: Urethritis is the most frequent single symptom of *Trichomonas vaginalis* and the one which brings the patient to seek medical advice. In the past many *Trichomonas vaginalis* urethritides have been labeled as non-specific urethritis. In their series Liston and Lees (18)

(1940) found that approximately 15% of males suffering from "non-gonococcal urethritis" were cases of *Trichomonas vaginalis* infestation.

c. Infestation of the Prostate, Seminal Vesicles, Bladder and Upper Urinary Tract: Fortunately these cases are rare but invariably whenever the invasion of these anatomical areas takes place, the symptoms and signs which follow are severe in nature. The symptoms are similar to those seen in the infection of these organs by other organisms.

#### THERAPY

1. *Local Therapy*: This form of therapy consisted mainly of topical application, local irrigation, and instillations. Different types of solutions were used *e.g.*, aqueous, acriflavine, hydrochloride, silver nitrate, mercuric chloride, mercuric oxycyanide and potassium permanganate. To these were added urethral soundings and prostatic massages and, in female patients, acid douches and administration of estrogen hormones.

Many powders and vaginal suppositories are used in the treatment of *Trichomonas vaginalis*. Among them is the combination of Furoxone (furzolidone) which was said to be bactericidal and a specific trichomonacide and Micofur (mifuroxime) a fungicidal nitrofurone effective against *Candida* (*Monilia*) *albicans*. This combination is known under the brand name of Tricofuron.\*

Following the advent of sulfonamide therapy and antibiotics, these were used in conjunction with topical therapy, soundings and prostatic massage.

Treatment lasted from six to twenty months with no improvement in the urethral discharge. Kleegman (38) (1930) observed a ten to thirty per cent recurrence rate after treatment of *Trichomonas vaginalis*.

The older topical treatment was not only painful for the patient but inadequate and many times potentially dangerous. Solutions were inadequate because they failed to reach the flagellates hidden in the folds and fissures of the mucosa inaccessible to the chemical [Faust (39) 1963]. The reported therapeutic "successes" have been simply temporary symptomatic relief, without evidence of a definitive cure as proven by a return of symptoms and by the demonstration of *Trichomonas vaginalis* in the discharge and secretions.

The reason for this failure was the presence of *Trichomonas vaginalis* in inaccessible sites, glands and ducts of the lower genito-urinary tract (Skene's ducts, Bartholin's glands).

2. *Systemic Therapy*: Many drugs have been used for systemic therapy in an attempt to eradicate *Trichomonas vaginalis* infestation. Most of these drugs belong now to the history of *Trichomonas vaginalis* therapy. Bactericidal and bacteriostatic drugs (sulfonamides, penicillin and other antibiotics) had no effect on *Trichomonas vaginalis*. An oral preparation with good trichomonacidal activity *in vitro* and in animals was Tritheon† (2 acetyla-

\* Eaton Laboratories, Norwich, New Jersey.

† Ortho Pharmaceutical Corp., Raritan, New Jersey.

mino- 5 nitrothiazole). In human trials, however, it did not give satisfactory results.

Recently a new trichomonacidal agent, Metronidazole (Flagyl)\* has been added to the medical armamentarium. In a monograph published March 16, 1963 (J.A.M.A., 183-11: 952-3) the A.M.A. Council on Drugs stated, "its effectiveness is greater than that of any other form of therapy used to date." Metronidazole was synthesized by the Research Department of Rhone-Poulenc, France. It has been used widely in Europe since 1959. Over fifty reports on its pharmacological and clinical action have been published in the medical literature and approximately 14 of these have been from the United States.

The present paper will summarize the studies conducted in the Vaginitis Clinic at The Mount Sinai Hospital and will describe the findings and results in a group of male patients treated with Flagyl.

#### EXPERIENCE WITH METRONIDAZOLE (FLAGYL) IN THE VULVO-VAGINITIS CLINIC AT THE MOUNT SINAI HOSPITAL

Perl and Ragazzoni (40) (1962) published a preliminary report on Flagyl dealing with 61 female patients attending the Vulvo-Vaginitis Clinic at The Mount Sinai Hospital in New York City. In 1963 Perl *et al.* (41) presented a review of a larger group of patients concluding that "Flagyl must be accepted today as one of the great advances of treatment of this much discussed and studied but unresolved problem."

Having recognized the importance of the male partner in the transmission of *Trichomonas vaginalis*, the senior author invited a urologist to co-operate in the study and treatment of male patients. In doing so we had two purposes in mind:

1. To determine the frequency of *Trichomonas vaginalis* infestation in the male population in general, and in the male partner of the infected female in particular;
2. To evaluate the effect of Flagyl in the male, mainly its effect on spermatogenesis. Having already determined that Flagyl was without side effects in female patients it was left to us to determine its influence on spermatogenesis.

#### METHODS OF STUDY

Our patients were referred to us from the Vaginitis Clinic as the partners of infected females, or from the Urology Clinic where they sought help for the treatment of a so-called "non-specific urethritis." Having ruled out other causes for their symptoms, the patients were investigated for *Trichomonas vaginalis*. Trichomoniasis in the male can manifest itself as an overt infection of the uro-genital tract, but many times the male acts simply as a healthy

\* Searle & Co., Chicago, Illinois.

carrier of the protozoa without symptoms. Therefore repeat examinations are necessary because the presence of the protozoa is overlooked in a significant percentage of male patients.

The method of investigation of the male patient for the presence of the trichomonas infection consists in a thorough history, physical examination and examination of a fresh semen specimen which has been ejaculated directly into a sterile jar.

We insist on having fresh specimens not older than twenty-four hours. When semen could not be obtained for one reason or another, a prostatic massage was performed instead and the prostatic fluid was examined and cultured. We found to our surprise that many times the prostatic fluid yielded *Trichomonas vaginalis* while the semen was sterile.

At the initial examination and at each subsequent visit the patient was examined for the presence of the protozoa by the wet smear method (using *Trichomonas Diluent*\*) and with the Kupferberg Simplified Trypticase Serum Culture Medium. The wet smear with *Trichomonas Diluent* is a highly sensitive diagnostic aid but in male trichomoniasis we depend finally on the culture method.

A thorough and complete semen examination was performed before and after treatment with Flagyl. Each specimen was examined for viscosity, motility, volume, count per cc, turbidity and morphology.

Part of the semen specimen was cultured using the STS Kupferberg Medium and incubated at 37°C. and readings obtained after twenty-four hours. If negative, the readings were repeated after forty-eight and seventy-two hours of incubation. If the specimen was positive for *Trichomonas vaginalis*, the patient was started on Flagyl therapy consisting of 250 mg tablets three times daily for ten days.

The patients were seen at the completion of the therapy and were evaluated for side effects. A repeat sperm specimen was obtained and cultured in the same manner as previously outlined. If negative, we saw the patient once more and only after two consecutive negative cultures was the patient considered cured.

#### CASE REPORTS

Following are five typical case histories of patients affected by *Trichomonas vaginalis*. These histories will illustrate the importance of examination and simultaneous treatment of the male partner of an infected female.

1. Mrs. N.F. was one of our severest cases of *Trichomonas vaginalis* vaginitis seen at the Clinic since 1953. She presented with recurrent acute flare-ups accompanied by urinary symptoms. Her husband had been treated in many urological clinics for active trichomoniasis with severe urethritis and urethral stricture. He reinfected his wife many times. During the nine years Mrs. F. attended our clinic we were able to bring on a clinical improvement

\* Ortho Co., Raritan, New Jersey.



or a transitory cure with different trichomonacidal compounds but each time she returned after sexual contact exhibiting all the acute symptoms of reinfection. In June 1961 we started treatment with Flagyl on Mr. and Mrs. F. They both became negative, and since then we have obtained seven negative cultures from the vagina and semen.

2. A.W. was treated for prostatitis since 1960. The prostatic fluid was negative for *Trichomonas vaginalis*. His semen, however cultured in S.T.S. culture media was positive for the protozoa. For two years he had been treated with all available chemicals but the semen remained positive for the protozoa and he reinfects his wife repeatedly. We gave him Flagyl in July 1962. Since then we have obtained four semen specimens which were all negative. His wife was cured as well.

3. Mr. N.J. was under treatment for prostatitis for four years. Mrs. J. was treated in our clinic during this time for recurring trichomoniasis. We had no knowledge of her husband's urological infections. When we cultured his first semen specimen we had the answer to the causative factor in the infection and reinfection. We treated them both with Flagyl for ten days and since then we have had four negative semen and vaginal cultures.

4. H.W. a healthy 43 year old married Negro had a urethral discharge lasting for about a year. Routine laboratory tests excluded gonorrhea and the patient was treated with different agents for a "non-specific urethritis." The patient did not respond to therapy and his urethral discharge continued. About the same time we found out that the patient's wife suffered from a profuse vaginal discharge. A culture established that she was affected by *Trichomonas vaginitis*. She was treated with Flagyl and cured. The patient had a positive semen culture for *Trichomonas vaginalis* and was given a full course of Flagyl. His urethral discharge subsided and two subsequent cultures were negative for *Trichomonas vaginalis*. The patient had no ill effects during this course of therapy.

5. J.H., a 25 year old colored male, had a sudden onset of urethral discharge and burning on urination. A gram stain was negative for gonorrhea and the patient received conventional treatment for a "non-specific urethritis," but did not respond to therapy. A semen culture disclosed *Trichomonas vaginalis*. The patient had a full course of Flagyl without any side effects. Two successive semen cultures were negative. Patient was discharged as cured.

#### RESULTS

A total number of 94 male patients were examined. Among the 94 individuals, 26 (28%) had a positive culture, while 68 (72%) were negative, as seen in Table I.

Among the positive *Trichomonas vaginalis* males, only 3 (12%) had symptoms, indicating how unreliable symptoms are in the epidemiology of *Trichomonas vaginalis*. 23 patients (88%) were positive for *Trichomonas vaginalis* and asymptomatic, as shown in Table II.

Twenty-one patients with positive *Trichomonas vaginalis* cultures received Metronidazole (Flagyl). All these patients became negative after treatment, as shown in Table III.

The drug has no ill effects on the spermatozoa. As we can see in Table IV there were no significant changes in the count per cc, volume, motility, morphology, viscosity or turbidity.

TABLE I

Total Number of Patients Examined	Total Number of Positive Patients	%	Total Number of Negative Patients	%
94	26	28	68	72

TABLE II

Total Number of Positive Patients	Total Number of Positive Patients with Symptoms	%	Total Number of Positive Patients without Symptoms	%
26	3	12	23	88

TABLE III

Number of Patients Positive for <i>Trichomonas Vaginalis</i> and Treated with Metronidazole (Flagyl)	Number of Patients Positive Treated Who Became Negative	%	Number of Positive Patients Treated and Still Positive
21	21	100	None

TABLE IV

*Comparative Studies of Semen Examinations Before and After Therapy with Metronidazole*

Count per cc:	No Change
Volume	No Change
Motility	No Change
Morphology	No Change
Viscosity	No Change
Turbidity	No Change

#### SUMMARY AND CONCLUSIONS

1. Local application of chemotherapeutic agents will not eradicate *Trichomonas vaginalis* from the female and male genito-urinary tract.

2. The cure of *Trichomonas vaginalis* necessitates the proper examination and simultaneous treatment of the male sex partner.

3. The male can be a symptom-free carrier of the protozoa and the organism can be detected from a freshly ejaculated semen specimen and/or prostatic fluid.



4. To evaluate the curative properties of a trichomonacide, cultures are essential.

5. Metronidazole (Flagyl) taken orally for ten days is a highly effective drug in the treatment of trichomoniasis.

6. Comparative studies of semen examinations before and after therapy with Flagyl show no ill effects on the qualitative or quantitative character of the semen and no depression of spermatogenesis.

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# Diagnosing Benign and Malignant Intracranial Disease with Mercury<sup>203</sup> Neohydrin Photoscanning

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## INTRODUCTION

Cerebral angiography and pneumography are reliable diagnostic modalities. These procedures, however, are not without danger or inconvenience. Furthermore, in many practice situations there is need for a relatively simple, accurate screening examination to detect intracranial space-occupying processes.

An alternate diagnostic approach has evolved from the application of radioactive isotope scanning techniques to brain disease (1, 2). Appropriate isotope compounds are available which will pool at abnormal sites by virtue of local "blood-brain barrier" breakdown. This disruption allows material usually excluded to remain focally where it has been detected in the past with varying degrees of accuracy.

Although isotope scanning has been known for several years, until recently methods and materials have been relatively crude. It is the purpose of this paper to describe newer developments in isotope scanning and to present techniques and experience derived from over 300 Hg<sup>203</sup> Neohydrin brain photoscans.

## INSTRUMENTATION

A good scanning device allowing maximum detection of counts from the target area and minimum detection from outside the target area will contain the following components (3):

A) A scintillation detector with high sensitivity. This is now available in the form of larger NaI crystals which can be adequately shielded and yet move easily.

B) Efficient focusing collimators with high resolution and uniform response to tumor radioactivity, relatively independent of the depth of the tumor in the organ scanned. True independence of response is achieved only by coincidence counting of positron emitting isotopes. This requires a rather expensive positron scanner which cannot be used for other diagnostic applications as can the conventional collimator scanner (4). Properly designed collimators are now available, however, which approximate independence of response, up to several inches in depth (5).

C) Highly stable pulse height analyzers. These must reliably maintain a constant voltage supply.

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D) A photographic method of data presentation. Previously, scan data was displayed on paper by a solenoid hammer which portrayed variations in spacing between uniform dots or lines. With this technique the small (10 per cent to 30 per cent) differences in count rate between tumor and normal tissue (6) encountered in brain scanning were not readily apparent.

On newer instruments, scanning data is led to a cathode ray tube whose variable glow makes a photographic image on ordinary x-ray film. Areas of increased count rate are represented by increasing degrees of film darkening. The film density-count rate relationship is arranged so that a slight increase in count rate produces a considerable increase in film blackening, *i.e.*, one obtains a high contrast, non-linear response over a limited range. On the resulting photosean, the eye can more readily appreciate small differences in count rates when presented as changes in film density.

#### ISOTOPE DEVELOPMENT

Various beta and gamma emitting isotope compounds for use in brain scanning have been tried in the past. The use of radioactive  $I^{131}$  tagged human serum albumin has recently been advocated by some (7, 8). Although unquestionably useful this compound has certain physical and biological disadvantages.

For example, although the major gamma energy of  $I^{131}$  is a 364 K.E.V. photon, there are significant amounts of two other higher energy gamma photons which can originate outside the target area reaching the detector by penetrating crystal shielding, crossing collimator septae or scattering into the field and losing just enough of their energy to be counted with the 364 K.E.V. peak. In practice, as much as one-half of the total count rate can come from outside the area under the collimator, markedly decreasing precision, regardless of the scanning instrument (6).

Furthermore, at scan time (24-48 hours after injection) 80 per cent of the  $I^{131}$  albumin is still in the body and 50 per cent remains in the blood. This results in a high body background which reduces tumor-nontumor ratios. Mass localization in the region of large blood vessels such as the superior sagittal sinus or transverse sinus is rendered increasingly difficult.

Recently,  $I^{131}$  labeled polyvinylpyrrolidone has been suggested for use in brain scanning. Experience with this compound is thus far limited but it may be a useful alternative to  $I^{131}$  albumin (8).

Mercury<sup>203</sup> linked to Neohydrin (chlormerodrin) is a more appropriate compound for use in brain scanning (6).  $Hg^{203}$  emits only a single 280 K.E.V. gamma photon. This minimizes scattering problems and simplifies shielding and collimation. It can be shown with phantom studies that for the same geometry, a "tumor" will produce 30 per cent more counts with  $Hg^{203}$  than with  $I^{131}$ , a practical improvement in target-nontarget ratio.

Indeed, even though  $Hg^{203}$  has a longer physical half-life than  $I^{131}$ , its actual biologic half-life, when linked to a diuretic such as Neohydrin, is much shorter than that of  $I^{131}$ . In comparison to the high level of background  $I^{131}$  albumin radioactivity present during scanning, about 80 per cent of  $Hg^{203}$

Neohydrin radioactivity has left the blood in 5 hours and over 95 per cent has left the blood in 24 hours (10). A bi-phasic  $\text{Hg}^{203}$  Neohydrin excretion pattern is present. Three-fourths of the administered dose is eliminated with an effective half-life of 5 hours while the remaining one-fourth is excreted with a half-time of 7 days (11).

A 70 kg man requires 350 microcuries of  $\text{I}^{131}$  albumin for a brain scan with resultant total body dose of 700 millirads. This may be compared to a required injection of 700 microcuries of  $\text{Hg}^{203}$  Neohydrin. Although this is twice the radioactivity of the  $\text{I}^{131}$  compound, a resultant total body dose of 290 millirads is received, less than one-half the total body dose obtained from  $\text{I}^{131}$ .

It must be pointed out that 10 per cent of the administered  $\text{Hg}^{203}$  Neohydrin localizes in the kidneys with a biologic half-life of 28 days and this will result in a dose of 35 rads to these organs. If one, however, gives an intramuscular dose of 1 cc of Mercurhydrin (meralluride) 24 hours before scanning, this apparently blocks sites of uptake in the kidneys (6). Renal dosage is decreased to 13 rads, an exposure which the kidneys might receive during a G.I. series. Practically,  $\text{Hg}^{203}$  with its half-life of 48 days, has a longer shelf-life than the shorter-lived  $\text{I}^{131}$  compound. Further, scanning is done a few hours after injection with  $\text{Hg}^{203}$  and patients are not required to return for 24 hour, 48 hour or 72 hour scans. When a group of patients are each scanned, first with  $\text{I}^{131}$  and then with  $\text{Hg}^{203}$ , a significant improvement in results is reported (6).

Soddee (12) has recently advocated the use of  $\text{Hg}^{197}$  Neohydrin in brain photoscanning. Its relatively short physical half-life (2.7 days) would further decrease renal exposure.

The use of positron emitting Arsenic<sup>74</sup> arsenate for detecting intracranial space-occupying processes has not found wide acceptance. In addition to cumbersome detector disadvantages, high doses of isotope must be used with correspondingly high total body doses. Further, tumor-normal brain concentration ratios for  $\text{As}^{74}$  arsenate are inferior to those obtained with either  $\text{I}^{131}$  serum albumin or  $\text{Hg}^{203}$  Neohydrin (13).

#### METHODS

A commercially available photoscanner (Picker MagnaScanner) is used incorporating a 3" x 2" NaI crystal and a 19 hole focused collimator (14). The pulse height analyzer is set between 250 K.E.V. and 300 K.E.V. The scan speed is 20 cm per minute and scan lines are .4 cm apart. A count per minute range differential (CRD) of 30 per cent is used. This means that photographic gradients from white to black will occur through the upper 30 per cent of the maximum number of counts. A higher CRD is used in selected cases. A time constant of one-fourth second is used. The "density" setting, which controls duration of cathode ray tube (CRT) glow in micro-seconds for each photon detected, is usually fixed at 100. Standard x-ray film is used and is manually developed at 68 degrees Fahrenheit for 5 minutes. Counts range between 200-1000 per minute.

Approximately 24 hours before scanning, the patient receives 1 cc of



Mercuryhydriin intramuscularly, for blockage of sites of uptake in the kidneys and dose reduction. On the day of the study, without any dietary restrictions, the patient receives an intravenous injection of 10 microcuries kg of  $\text{Hg}^{203}$  Neo-hydriin. The maximum amount given to any patient does not exceed 700 microcuries. No side effects have been reported in the literature nor have any been observed in our own series. Although varying time intervals have been tried and are still being tried, we now begin scanning 3 hours after injection. This allows sufficient time for additional scan positions and confirmation of questionable areas without the necessity of additional isotope and is, in our opinion, sufficient for visualization of abnormalities. In clinically suspected vascular malformations, however, scanning is begun 1 hour after administering the isotope.

The patient's head is immobilized. The initial scan position is an appropriate lateral if localizing signs are clinically present. This is followed by either an A P or P A scan and then, if warranted, the opposite lateral position scan. In general, therefore, scans in at least three positions are obtained.

Total time for the study is about one and one-half hours. Young children or patients who cannot remain relatively quiet for this period of time are sedated. A dot scan, simultaneously accomplished with the photosean, is performed with background cutoff of 10 per cent and a dot factor of 2 or 8. The resulting low contrast dot scan supplements the high contrast photosean.

#### THE NORMAL BRAIN PHOTOSCAN

On the lateral scan (Fig. 1), the brain appears as a generally light area surrounded by a peripheral margin of activity which outlines the head contour. This rim of radioactivity-density is mainly due to concentration in scalp vasculature and in the superior sagittal sinus under the calvarium. The activity rim usually widens posteriorly, possibly due to the entrance of large superficial cerebral veins into the midline vascularity.

Four standard anatomic points are marked on the lateral photosean: the nasion, the external auditory meatus, the external occipital protuberance and the superior Rolandic point, the latter marking the approximate upper border of the Rolandic fissure. This point is found by halving the distance from nasion to occiput as measured with a piece of string and marking about 1 inch posterior to this halved distance. A line from this point to a point just anterior to the external auditory meatus will outline the Rolandic fissure and is most helpful in anatomic localization. There is always marked increase in activity below a line extending from the nasion to the external auditory meatus due to increased pickup in orbital and temporal muscle vasculature. Variable activity is normally present below a line connecting the external occipital protuberance and the external auditory meatus due to lateral dural sinus size variations. Except for this, both lateral scans should be symmetrical.

On the antero-posterior scan (Fig. 2) a central area of low activity representing brain tissue is encircled by a rim of increased radioactivity-density due primarily to scalp vasculature. Extending down from the vertex in the midline is a band of increased density from central vascular structures. Photoscan marks are placed at the vertex, nasion and outer-canthi. Below the nasion area, a constant zone of increased activity is present, rarely asymmetric, due to radioactivity pickup in oculo-facial muscles, in paranasal sinus vascular mucous membrane and in tongue and neck musculature.

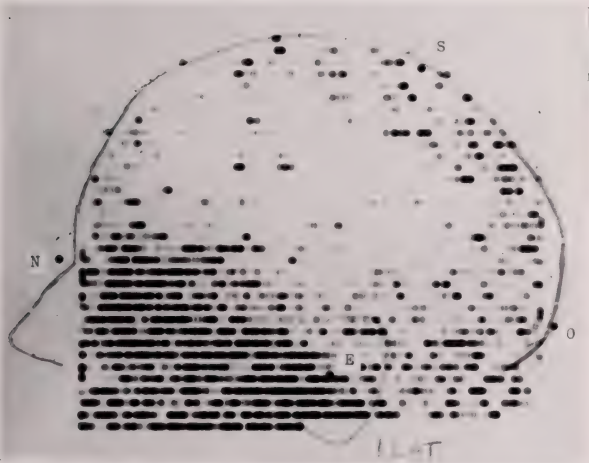


FIG. 1. Normal lateral photoscan. Note peripheral vascular rim increasing posteriorly, density in facial area, variable density in posterior fossa. N (nasion), S (superior Rolandic fissure), O (occipital protuberance), E (external auditory meatus).

On the postero-anterior scan (Fig. 3) a peripheral rim of increased density encircles the low activity normal brain tissue. A midline band of increased activity due to vascular structures extends from the vertex, where it is heaviest, to the external occipital protuberance. From the latter point to the mastoid tips, marked in the photoscan, there is a normal constant increase in activity, having the form of an inverted "V." When posterior fossa lesions are being searched for, the probe is angled about 15 degrees, and a "reverse Towne" scan is performed. This is generally similar to the postero-anterior scan in appearance with the exception that the normal area of increased radioactivity in the occipital region has a relatively horizontal upper margin, rather than an inverted "V" configuration.



## THE ABNORMAL SCAN

On an abnormal scan, a striking zone of increased radioactivity-density is perceived in an area not usually containing such a pattern (Fig. 4). A neoplastic mass will generally be rounded and will vary in size from the limits of resolution (about 2.5 cm) to many centimeters. It may be homogeneous or variable in density. Because of inherent contrast enhancement



Fig. 2. Normal anteroposterior photoscan. Note peripheral vascular rim, increasing density in facial area. V (vertex), N (nasion), C (outer canthi).

in the scanner circuitry, an abnormal area often tends to stand out and normal vasculature may be suppressed or not depicted (Fig. 5). This is especially true when a marked difference is present between tumor count rate and the number of counts in normal brain structures. The explanation is that the photographic display from white to black occurs in the upper 30 per cent of the maximum number of counts for the usual "CRD" of 30 per cent. Thus, if a relatively "hot" tumor produces a maximum number of counts per minute equal to 1000, then all areas with less than 700 will not be depicted on the photoscan. These areas are the normal vascular portions of the brain

which usually give no more than 200-300 counts per minute. The resulting scan is very striking but obviously tumor not differing greatly from normal structures in count rate would be missed.

Practically, this may often be avoided with a routine preliminary "hand scan" of the brain, before the automatic scanning motor is started. If no obvious "hot spot" is found when the probe is moved over the brain, the



FIG. 3. Normal posteroanterior photosean. Note increased midline vascular density, peripheral rim, inverted "V" appearance in occipital area. O (occiput), M (mastoid tips), V (vertex).

maximum number of counts should be selected in an area away from the normal high count zones which are found over temporal-facial and occipital musculature and posterior to the vertex. This increases the possibility of depicting low count rate tumors. Appropriate lateral and coronal scan positions are most important in this type of neoplasm (Fig. 6).

Another approach to the low count neoplasm and to brain scanning in general involves utilization of high "CRD" with a resultant low contrast scan. The latter is then viewed on closed circuit television, where contrast may be varied. It is our feeling that this is not necessary since we routinely



FIG. 4. Abnormal radioactivity-density in right parieto-occipital region. Note normal vascular, facial densities. Glioblastoma.



FIG. 5. Abnormal frontoparietal radioactivity-density. "High count rate" tumor pattern with suppression of normal vascular, facial densities. Meningioma.

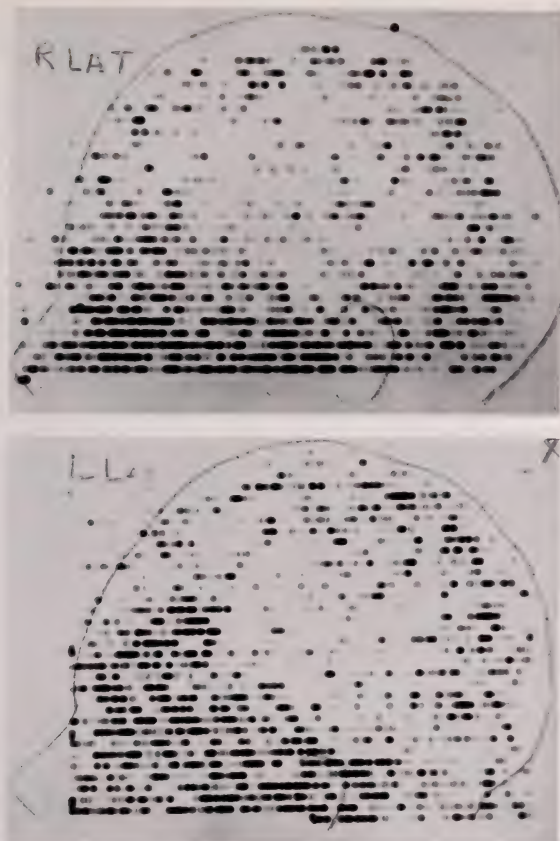


FIG. 6. (A) (Lower) Left lateral scan. Definite "low count rate" tumor pattern in anterior frontal region. Metastatic adenocarcinoma. (Upper) Right lateral scan. No definite abnormality. Only appropriate lateral portrays tumor.

partially re-scan problem areas with variation of the contrast "CRD" setting.

If a "hot spot" is found on the preliminary manual "hand scan" one can be certain of an abnormality, although photoscanning is performed to obtain a permanent record and for precise localization.

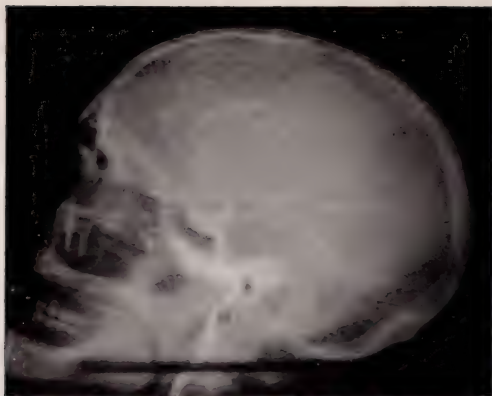


FIG. 6. (B) Left lateral cerebral angiogram. No detectable abnormality.

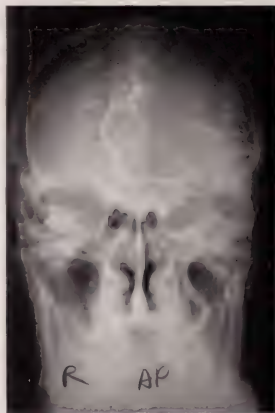


FIG. 6. (C) Anteroposterior scan shows density in left frontal region confirming finding on left lateral scan. Left-sided anteroposterior angiogram shows anterior cerebral artery shift.

#### AUTHOR'S MATERIAL

Scans were performed as an office procedure on 319 patients referred largely by neurosurgeons and neurologists. All were suspected of having intracranial space-occupying processes and ranged in age from 21½ years to 81 years.

Eighty-one patients were proven to have localized intracranial disease at surgery, autopsy or on the basis of clinical course and other diagnostic studies. Sixty-three of the group had neoplasms and 18 had non-neoplastic disease (Table I). Seventy of the entire group or 87 per cent had localizing positive scans. Fifty-four of the neoplastic group or 85 per cent had positive localizing scans. Cerebral angiography was performed in 42 of the 63 tumor patients and was abnormal in 39 or 93 per cent. False negative angiograms were obtained on one patient with a parieto-occipital glioblastoma, another with

TABLE I  
*81 Patients with Localized Intracranial Disease*

Diagnosis	Scans Positive	Scans Negative
<b>I. Neoplastic Group</b>		
Meningioma .....	8	
Glioblastoma .....	18	1 (temporal, invading floor of third ventricle)
Metastatic.....	18	3 (1 diffuse, 1 temporal, 1 parietal tumor in cyst)
Astrocytoma.....	4	
Glioma (Unspecified).....	4	1 (patient uncooperative)
Ependymoma.....		1 (posterior fossa, child)
Pituitary.....		2
A-V Malformation .....	2	
Acoustic.....		1
Neoplastic Group Total.....	54	9
<b>II. Benign Group</b>		
Subdural Hematoma.....	4	
Intracerebral Hematoma, Aneurysm.....	3	1 (temporal)
Intracerebral Infarct.....	2	
"Cerebrovascular Accident".....	4	
Middle Cerebral Thrombosis .....	2	
Internal Carotid Thrombosis.....	1	
Intracranial Tuberculoma.....		1
Benign Group Total.....	16	2

a posterior parietal astrocytoma, a third with a low fronto-temporal glioblastoma and a fourth with a large occipito-parietal cerebral infarct. In these anatomic locations space-occupying processes may not easily reveal themselves on angiography. These patients, however, had positive localizing scans. For technical reasons in 8 positive angiograms the abnormality was lateralized only without precise localization. In 6 of these 8, scanning accurately portrayed the lesion.

Pneumoencephalography or ventriculography was performed on 6 patients in the tumor group and localized the lesion in each case, including 3 with falsely negative cerebral angiograms. Three of these 6 patients had negative scans.

It is evident from Table I that 100 per cent of meningiomas, 94 per cent of glioblastomas and 85 per cent of metastatic foci gave positive scans.

Of 18 positive metastatic scans, 14 arose from squamous bronchogenic carcinoma, 2 from breast adenocarcinoma, 1 from malignant melanoma and 1 from reticulum cell sarcoma. Of 3 false negative metastatic scans, 2 were from breast primaries and 1 spread from the lung.

Our series of 18 non-neoplastic disease processes consists of 1 intracranial tuberculoma, 4 chronic subdural hematomas, 2 middle cerebral artery thromboses, 1 internal carotid thrombosis, 4 intracerebral hematomas, and 2 brain infarcts. Four cerebrovascular accidents are also included in this group. Positive scans were obtained in all but two of these cases, (excluding a large number of "CVAs"). The positive scans apparently result from "blood-brain barrier" disruption occurring under some conditions secondary to intracranial vascular disease.

Cerebral angiography was not performed on the brain infarct and "CVA" patients but clinical and laboratory features were diagnostic. It is a matter of interest that one positive brain infarct scan had reverted to normal when repeated after three months, and that one positive scan on a "CVA" patient receded in 4 weeks. Arteriograms performed on the remaining 10 patients in this group were diagnostic.

Ten falsely negative scans were obtained and these are listed with comments in Table I. The false negative rate is seen to be 15 per cent in the tumor group, 6 per cent in the benign categories (excluding "CVAs"), 13 per cent in the entire positive scan series. The remaining 238 negative scan patients were clinically followed for periods ranging from 6 months to 2½ years and in the opinion of attending neurologists and neurosurgeons were felt to have no localized intracranial disease. Most were diagnosed as having one of the following abnormalities: epilepsy, cerebrovascular accident, generalized convulsive disorder, hypertensive encephalopathy, conversion reaction, Guillain-Barré Syndrome, Alzheimer's Disease, multiple sclerosis, encephalitis.

#### DISCUSSION

There is a marked pickup of Hg<sup>203</sup> Neohydrin associated with glioblastomas, meningiomas and metastases. Obvious "hot spots" are detected and striking scans are produced depicting dense abnormalities in coronal and lateral positions. Our experience generally coincides with other reported series (15, 16).

In evaluating the significance of possible tumor densities the following principles have been found useful: tumor radioactivity-density will be localized in a region not normally showing an increased count rate. A radioactivity-density must persist in several adjacent scan lines seen in at least 2 perpendicular scan positions.

Scans will be seen, however, in which densities are consistently depicted with disproportionate blackness in one scan position compared to another. In our experience, this strongly suggests non-neoplastic space-occupying



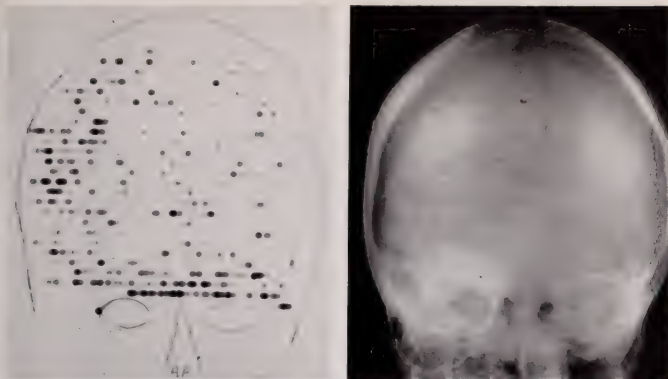


FIG. 7. (A) (Left) Abnormal density in right convexity region. (Right) Right cerebral angiogram showing chronic subdural hematoma.

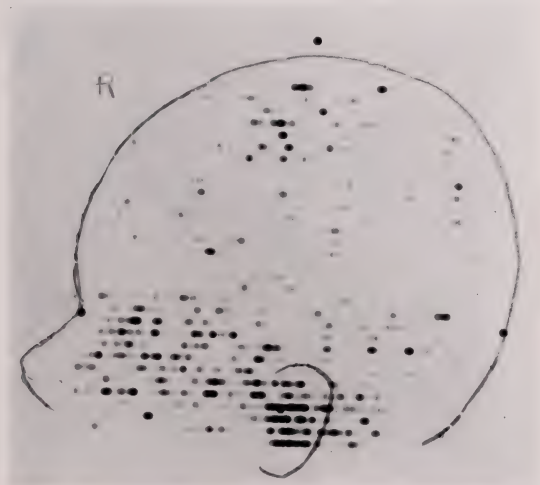


FIG. 7. (B) Right lateral scan. Small density in frontoparietal region. Compare to disproportionate density of anteroposterior scan. (Fig. 7. (A))

disease. Data in the latter category are thus far quite limited (16, 18). It would appear, nevertheless, that an as yet unknown fraction of chronic subdural hematomas, acute internal carotid thromboses, middle cerebral artery thromboses, intracerebral hematomas, brain abscesses and brain infarcts will give positive scans (Figs. 7, 8). At least 1 positive brain infarct scan and

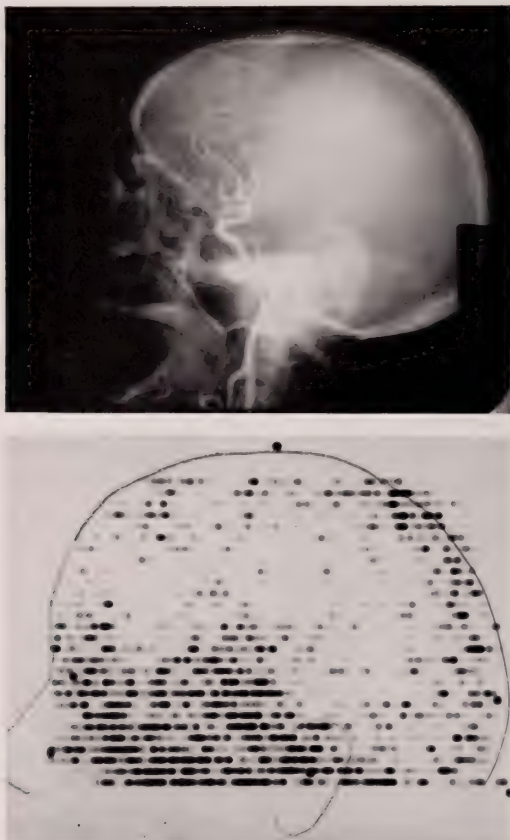


FIG. 8 (Lower) Right lateral scan. Abnormal temporal density. Anteroposterior scan showed corresponding abnormality but of lesser density. (Upper) Right lateral cerebral angiogram showing partial thrombosis of middle cerebral artery.

1 positive CVA scan have reverted to normal. An evaluation of  $\text{Hg}^{203}$  photo-scans in non-neoplastic space-occupying processes will be presented in a future communication.

Certain limitations are apparent. With present instrumentation, it is physically unlikely that lesions smaller than 3 cm will be detected. Space-occupying processes in certain sites have not been readily portrayed. This is true in the parasellar region and in the posterior fossa. In both these areas, radio-

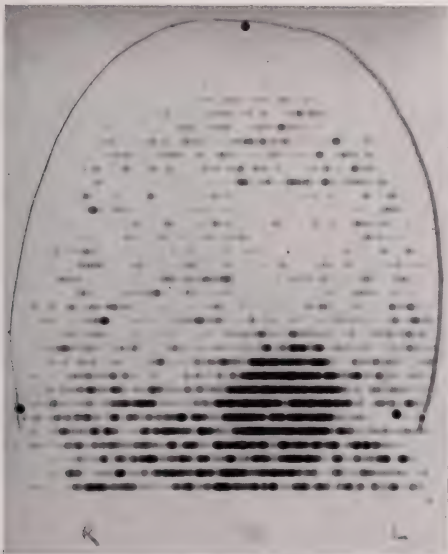


Fig. 9. Posteroanterior scan. Study made four years after occipital craniectomy for removal of posterior fossa meningioma. Increasing symptoms. Radioactivity-density left occipital region. Left lateral scan revealed density in anterior portion of posterior fossa. Recurrence found at surgery.

activity-density is normally present in sizeable amounts and identification is difficult unless huge or very "hot" lesions are present which project a distance of several centimeters. Of our missed diagnoses, 4 were in the parasellar area and 1 was in the posterior fossa.

The histology of some lesions renders detection difficult. For example, well differentiated astrocytomas, cystic tumors, craniopharyngiomas, pituitary adenomas, pinealomas, cerebellar ependymomas and medulloblastomas, acoustic neuromas and certain metastatic foci are not often successfully scanned.

However, the fact that these tumors generally emit perhaps only a few hundred net counts per minute more than normal brain vascular background allows one to exclude meningiomas, glioblastomas or metastases when a "low count" density is significantly detected in 2 perpendicular scanning positions. Although histologic diagnosis cannot definitely be made one could reasonably suggest an unusual neoplasm such as a well differentiated astrocytoma.

In our experience, preceding angiography or pneumography have had no detectable effect on the  $\text{Hg}^{203}$  scan. Others (3, 15), however, have reported interfering abnormal areas of uptake after these studies.

Craniotomy will definitely result in increased areas of radioactivity-density

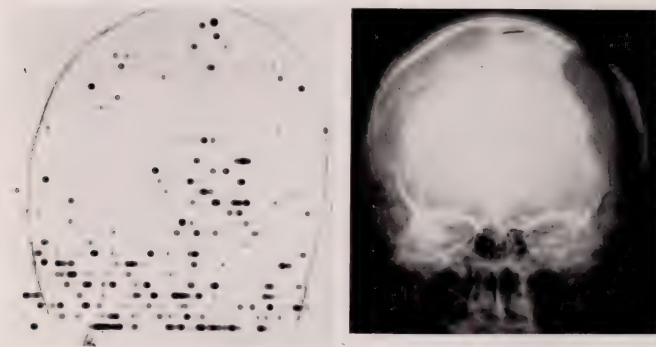


FIG. 10. Posteroanterior scan made nine months after decompression craniotomy. Recurrent symptoms. Note deep tumor density on left. Lateral scan showed similar density in parietal region. Note elevated flap on skull film. Low grade astrocytoma.

at the bone flap site, presumably related to marked "blood-brain barrier" disturbance at the time of surgery. The increased pickup will usually persist no more than 4-6 months but may occasionally remain for years. This phenomenon has provided a useful but simple follow-up technique in postoperative patients. An area of increasing density, either at the flap site or deep to it, compared to a postoperative base line scan, has correlated clinically with persistent tumor growth or recurrence (Figs. 9, 10).

Radiotherapy directed to the cranium will not provide sufficient "blood-brain barrier" disruption to result in positive photo-scans where the pre-therapy scan was negative. Certain toxic states (17) such as hepatic coma, drug intoxication and uremia, may alter the "blood-brain barrier" with resultant scan abnormality. In uremia, for example, a diffuse increase in normal brain vascular uptake will occur on the scan (Fig. 11), which may

in part be related to delayed renal excretion. Paget's disease gives a somewhat similar picture, presumably because of localized hypervascularity.

Despite limitations,  $\text{Hg}^{203}$  Neohydrin brain photoscanning is an advantageous diagnostic procedure. The study is innocuous and may be performed as an out-patient or office examination. Indeed, all of the scans in our series were performed in a private radiology office. Although scanning may be accomplished without the constant presence of a physician, he must be available to evaluate each scan and decide on modifications. Performance of the study

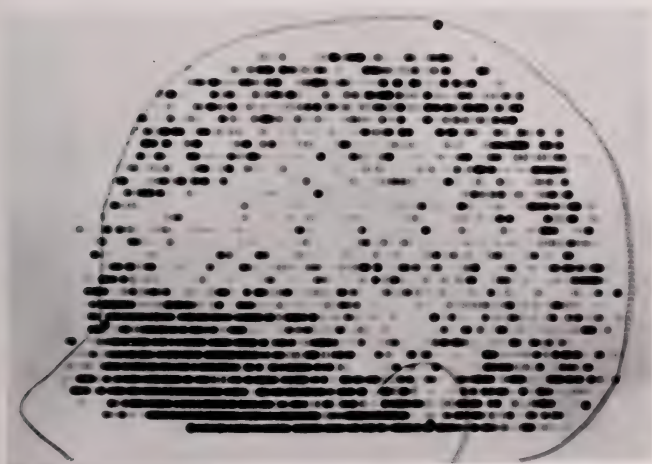


FIG. 11. Marked increase in peripheral vascular rim, facial density. Uremia.

must not be abandoned to a technician, especially if smaller or less radioactive lesions are to be detected.

An abnormality is usually depicted in its true size and location rather than indirectly localized by secondary deviation of vessels or ventricles from disease and adjacent edema. As a corollary, in several cases, although angiography demonstrated a tumor and its vascular supply, scanning was more informative for flap placement (Figs. 6, 12).

As yet, we have encountered no false positives nor have any been reported. It is to be expected that such cases will eventually be uncovered as photoscanning gains in popularity but the actual incidence must be small.

In our experience,  $\text{Hg}^{203}$  brain photoscanning approaches angiography and pneumography in diagnostic localization reliability. It is not, however, to be

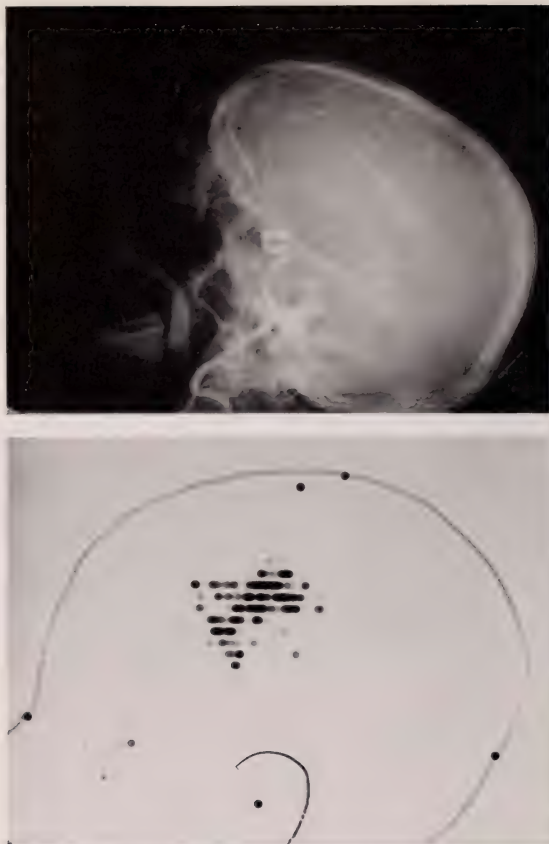


FIG. 12. (A) Left lateral scan shows tumor density in frontoparietal region. Left lateral cerebral angiogram is not specific.

considered a substitute for these studies since it is apparent that positive scans will be obtained in non-neoplastic space-occupying processes. At least some of the scans in the latter category will revert to normal. Brain photoseanning in practice, is used as the initial radiologic diagnostic procedure after plain skull films. Rarely, it has been the only study obtained before neurosurgery.



By comparing pre-operative and post-operative scans, the follow-up of patients after surgery and radiotherapy has been simplified. The diagnosis of metastatic intracranial disease in patients with known extracranial primary neoplasms has also been made less onerous.

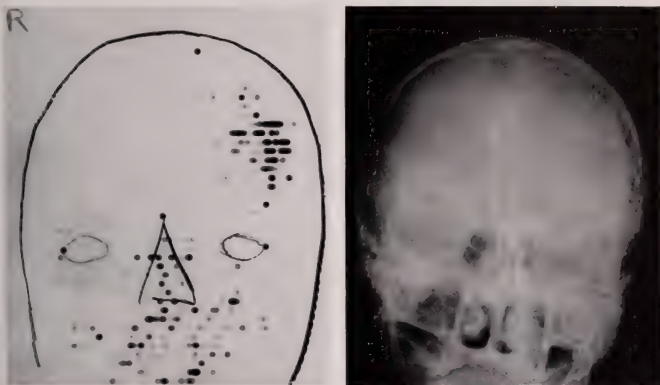


FIG. 12. (B) Anteroposterior scan shows corresponding relatively superficial tumor. Left anteroposterior cerebral angiogram reveals slight shift of anterior cerebral artery. Scans used as guide for bone flap. Glioblastoma.

#### SUMMARY AND CONCLUSION

Recent developments in scanning devices are discussed, together with physical, biological and practical advantages of  $\text{Hg}^{203}$  Neohydrin for use in brain abnormality detection. A method of photoscanning with this compound is described in detail. Characteristics of normality, neoplastic and non-neoplastic processes are discussed, based on the authors experience with 319 cases, including 63 tumors and 18 benign cases. Advantages and limitations of the procedure are considered. Mercury<sup>203</sup> Neohydrin brain photoscanning is recommended as a valuable tool in the diagnosis and follow-up of patients with intracranial disease.

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## RISA Brain Scanning

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### INTRODUCTION AND HISTORICAL BACKGROUND

Widespread experience with brain scanning over the past decade has shown that radioisotope techniques are of value in the diagnosis of intracranial disease. Although neoplasms are the most commonly detected intracranial lesions, the presence of hematomas, abscesses, and arteriovenous malformations can be established by brain scanning as well. A variety of other conditions not actually associated with the presence of a mass, in particular lesions secondary to occlusive cerebrovascular disease, may also be detected by means of radioisotope examinations. (1-7)

Brain scanning is relatively innocuous\*. Unlike angiography or pneumoencephalography, examinations performed with radioisotopes permit the clinician to gather valuable information without fear of complications. No special precautions are necessary, and hospitalization of the patient is not required.

George Moore first introduced the use of radioisotopes for the diagnosis of intracranial disease in 1947 (8). He had originally made the observation that brain tumors and certain other pathological tissues would selectively take up and retain the dye substance sodium fluorescein, permitting him to identify brain tumors at surgery by their fluorescence on exposure to ultraviolet light. Exploiting this knowledge, Moore set about to diagnose the presence of intracranial lesions by purely external means. He tagged fluorescein with a gamma-emitting radioisotope and administered the substance intravenously, recording the radioactivity over symmetrical regions of the patient's head with a Geiger-Mueller counter. He interpreted an asymmetrical increase in radioactivity as an abnormality.

By 1951, with somewhat improved instrumentation, Moore reported that he had correctly localized seventeen of twenty-six verified brain tumors; however he also diagnosed a number of normal cases in his series as having abnormal radioisotope studies (9). Brain scanning was introduced at The Mount Sinai Hospital in the same year, but because the method was found to give unreliable results, the procedure was discontinued within two years.

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\* Five microcuries per kilogram of RISA gives a whole body radiation dose of approximately .6 rad, for instance.

Although some early investigators reported a high degree of success with radioactive diiodofluorescein (DIF), subsequent studies by other investigators confirmed that optimum localization of abnormal concentrations of radioactivity could not be satisfactorily achieved with methods and materials then available (10, 11).

The successful refinement of detection and recording devices to automatic instruments of high sensitivity and reliability together with the introduction of more suitable tracer substances has served to change this original impression and to bring about a resurgence of interest in brain scanning (6).

#### METHODS AND MATERIALS

This report is based on the brain scans of 461 patients suspected of having intracranial lesions who were referred for radioisotope examinations by staff members of The Mount Sinai Hospital between December 3, 1963, and January 18, 1965. Forty-three follow-up studies were also performed on these patients during the same period. All the studies were made with radioiodinated human serum albumin (RISA).

RISA was introduced for the detection and localization of intracranial lesions in 1951 (12). It was found to be superior to DIF because of its longer biological half-life and its tendency to accumulate in diseased tissue in increasing amounts over a period of hours and days.

Patients received 5 cc/kg of RISA intravenously and were scanned both immediately following the administration of the tracer agent and again after 48 hours. A few patients were scanned only at 24 hours. Lugol's solution was administered orally both prior to and during the examination period in order to block thyroidal uptake of unbound radioiodine.

Patients were scanned both in the anterior and lateral positions. Patients who could tolerate lying on their chests were scanned in the posterior position when clinically indicated. Scans were made with a Picker Magna-Scanner II equipped with a three inch crystal and a photorecorder.

A fairly constant counting rate of from 600 cpm to 1,000 cpm was obtained over the nasion of patients in the anterior position. There was only a slight variation between the counting rates at the time of the immediate and delayed scans. The counting rate over the nasion was utilized to set the intensity of the photo scan, serving to make the area of the nasion the darkest area on the photo scan in most cases.

Landmark dots were placed over the nasion and over the tragus of the ear with the patient in the anterior position. In the lateral position, the nasion, external auditory meatus, and theinion were used as landmarks. In the posterior position, the middle of the rim of the foramen magnum and the mid position of the pinna were used as the reference points (Fig. 1).

Scrupulous care was taken to prevent rotation of the head in order to obtain true anterior-posterior and lateral positioning. Pressure tape was applied across the forehead and across the chin to immobilize the head. Patients were urged to doze or sleep when possible. Sedation was employed

only on rare occasions. The length of the examination was anywhere from one hour to an hour and one half. Additional views lengthened the scanning time accordingly.

#### THE NORMAL RISA BRAIN SCAN

Scans performed immediately following the administration of RISA reflect the essentially intravascular localization of the tracer agent at that time. The

THE MOUNT SINAI HOSPITAL  
NEW YORK

RADIO-ISOTOPE  
BRAIN SCAN REPORT

	Name/Sex/Age
	Location Hosp. No.
	Attending Physician

ISOTOPE:  
AMOUNT INJECTED:  
DATE INJECTED:

DATE(S) SCANNED:  
SCAN NUMBER:  
DATE OF REPORT:

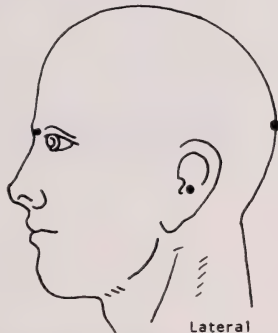
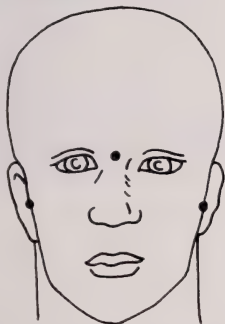


Fig. 1. Report form illustrating anterior and lateral scan positions with landmark dots. All lateral scans are shown facing to the right as in the diagram.

major vascular channels of the brain and the soft tissues at the base of the skull are visualized as dense concentrations of radioactivity (Figs. 2A, 2B).

In anterior or posterior scans, the superior sagittal sinus is clearly delineated at the vertex of the skull. The middle cerebral complex lying in the Sylvian fissure can be visualized approximately midway along the convexity of either cerebral hemisphere where there is a relatively focal increase in the amount of radioactivity present. There is a relative paucity of radioactivity over the more central portions of the hemispheres when compared with the convexities. The dense radioactivity at the base of the

skull in either the anterior or posterior view is often asymmetrical in the normal scan. The dominant lateral sinus, usually the right, can be made out on the posterior view.

The normal immediate lateral scan again demonstrates the superior sagittal sinus rather prominently, this time as it courses posteriorly, increasing in intensity as it receives major contributions from draining cortical veins. The confluence of great veins is visualized midway between theinion and the external auditory meatus. The more central portions of the lateral hemispheres as well as the structures of the posterior fossa below the torcular again reveal a relative paucity of radioactivity. Midway between the nasion and the external auditory meatus a focal concentration of radioactivity representing the vasculature of the Sylvian fissure, this time seen on lateral view, extends upward from the base of the skull in the shape of a pyramid.

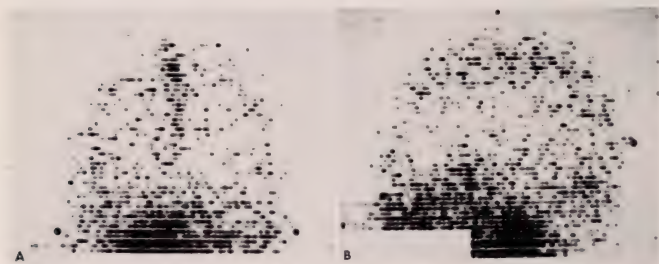


Fig. 2. Normal immediate (A) anterior and (B) lateral scans.

The base of the pyramid merges with the very dense radioactivity at the base of the skull itself.

Normal scans performed 24 or 48 hours after administration of RISA demonstrate subtle changes when compared with the more immediate studies (Figs. 3A, 3B). Although the vascular channels still dominate the scan, the relative intensity of radioactivity in these structures is not as striking as on the immediate scan. On either anterior, posterior, or lateral views, the vessels of the Sylvian fissure are not as easily made out, but the cerebral convexities still reveal a slight, but definite increase in activity when compared with the more central portions of the cerebral hemispheres themselves.

#### THE ABNORMAL BRAIN SCAN

The concentrations of radioactivity visualized on the normal brain scan have been shown to correspond to the vascular network of the brain and the soft tissues about the skull. Normal brain tissue itself takes up relatively little of the tracer agents commonly used for brain scanning. On the other

hand, the uptake of tracer substances by pathologically altered tissues in the brain is increased. Although the exact mechanism whereby tracer substances migrate into tumorous or diseased tissue is unknown, the net effect is to produce a concentration of radioactivity in a location where no such concentration normally is found to occur. Abnormal concentrations of radioactivity may be visualized as discrete or diffuse lesions, depending upon the nature and volume of pathological involvement.

#### THE CLINICAL IMPORTANCE OF BRAIN SCANNING

Brain scanning is most useful as a screening procedure in the evaluation of patients suspected of harbouring intracranial disease. It is particularly valuable in cases where clinical signs and symptoms do not indicate clearcut involvement of one side of the brain or the other.

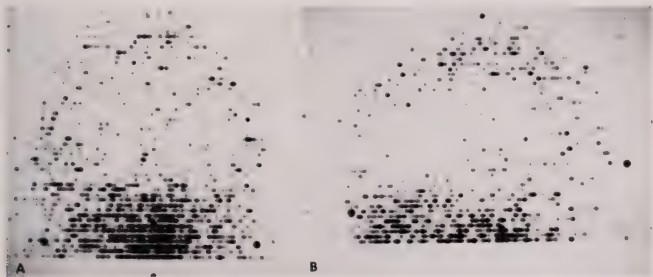


FIG. 3. Normal delayed (48 hr.) (A) anterior and (B) lateral scans.

Since the entire brain can be examined at the time scanning is performed, clinically unsuspected areas may be implicated as pathological. Scanning may not only lateralize disease, but may demonstrate that multiple areas exist.

The following case history will illustrate the role that brain scanning played in the diagnosis and management of a patient with a clinically unlocalized neurological picture.

A 65 year old man presented with confusion and memory loss. There was no evidence of focal neurological disease, and the patient was otherwise in good health. Blood chemistries and x-rays of the chest and skull were normal. An EEG indicated a diffuse abnormality. The CSF revealed a moderately elevated protein content. A right brachial angiogram was considered to be within normal limits.

A brain scan demonstrated the presence of two lesions, one in the left frontal region and the other on the left side of the posterior fossa. (Figs. 4A,B)

A left carotid angiogram confirmed the presence of an avascular mass in the left frontal region. Surgery was proposed, but in view of the findings of the brain scan, a pneumoencephalogram was performed. This study confirmed the presence of the second lesion on the left side of the posterior fossa.



A presumptive diagnosis of metastatic disease was made, and the patient was treated with radiotherapy.

Repeat radioisotope and contrast studies six weeks later demonstrated a slight decrease in the size of both lesions.

Brain scanning is a benign procedure and can be performed in situations where more rigorous testing is precluded by the patient's clinical condition, or where the index of suspicion is not sufficiently high to warrant the exploratory use of contrast studies to rule out the presence of demonstrable intracranial disease.

Because of the high incidence of neoplasms and other types of mass lesions in patients with abnormal radioisotope studies, positive scans are usually followed by contrast studies which may help elucidate the nature of

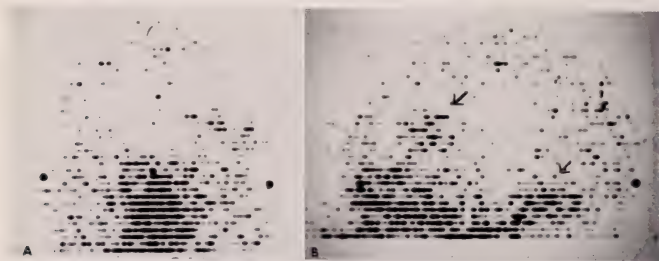


FIG. 4 48 hour (A) anterior and (B) left lateral scans in a patient with lesions in the left frontal region and left posterior fossa (arrows). Contrast studies were confirmatory.

the abnormality visualized by brain scanning. A positive scan may be the only indication that further diagnostic testing will be worth the risk.

The following case history is illustrative since a diagnosis would probably not have been readily established without the use of the brain scan:

A 54 year old woman was hospitalized complaining of nausea and vomiting. Seven years previously, she had undergone removal of an acoustic neuroma. Aside from her complaints, there was no evidence on neurological examination to suggest regrowth of the tumor. When the patient improved dramatically on bedrest and intravenous fluid therapy, her physicians were loath to repeat the contrast studies which had led to her original operation.

A brain scan was performed which demonstrated a lesion on the right side of the posterior fossa, the site of her prior pathology. (Fig. 5A).

In view of the brain scan findings, it was decided to perform a small posterior fossa air study to rule out regrowth of the tumor. The findings of the PEG were abnormal, confirming the brain scan (Fig. 5B).

At operation, a large, cystic, recurrent tumor was removed.

The most unique use of brain scanning is for follow-up studies. Scans performed over a period of weeks or months may be particularly valuable in



the management of cases where the diagnosis is inconclusive. Since hospitalization is not required, and since most patients have no objection to repeated radioisotope examinations, studies are easily accomplished on an out-patient basis. The level of radiation exposure can be held within such limits that no ill effects will result.

Brain scanning may indicate a focal or a diffuse abnormality during the more acute phase of an illness suspected of being due to cerebro-vascular disease. In-hospital studies such as angiography may demonstrate the occlusion of a blood vessel in some cases, yet in other cases contrast studies

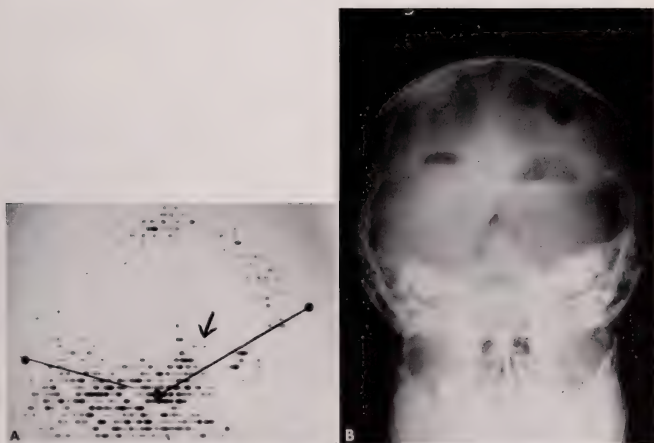


FIG. 5. (A) 48 hour right lateral scan in a patient with a recurrent acoustic neuroma (arrow). (B) Pneumoencephalogram in the same patient.

may demonstrate no more than evidence of a generalized arterio-sclerosis, and the patient may remain a tumor suspect. The disappearance of the abnormality visualized on the scan may help rule out the presence of a mass lesion.

Scanning may be performed as a follow-up measure in patients who have undergone neurosurgery or who have had radiation therapy. Recurrence or persistence of an abnormal scan may be of prognostic value in the management of patients, particularly when the clinical picture is misleading.

Two patients with abnormal brain scans and confirmatory contrast studies were followed by repeated brain scanning. One patient was treated with radiation therapy and became asymptomatic. The EEG also returned to normal. Repeat scan during this period demonstrated the lesion as seen on the original scan. The patient deteriorated subsequently. A metastatic lesion was found in the appropriate location at the time of necropsy.

The second patient improved markedly without any therapy at all and became asymptomatic. The diagnosis of an intracranial lesion was in doubt clinically despite the fact that her in-hospital studies were abnormal. Repeat scan again confirmed the lesion as originally seen. The patient soon succumbed, and at necropsy a glioblastoma was found.

Radioisotope studies may be indicated even after the presence of a lesion has been demonstrated by more conventional studies. Localization of intracranial lesions by means of brain scanning may often be superior to the results achieved by means of angiography or pneumoencephalography.

In angiography, the location of the pathological process must be deduced from the displacement of normal vascular structures in the absence of a "tumor stain" or conglomeration of abnormal blood vessels. Localization of a lesion by means of pneumoencephalography is dependent on the displacement of the gas-filled ventricular system, cisterns, or sulci from their normal posi-

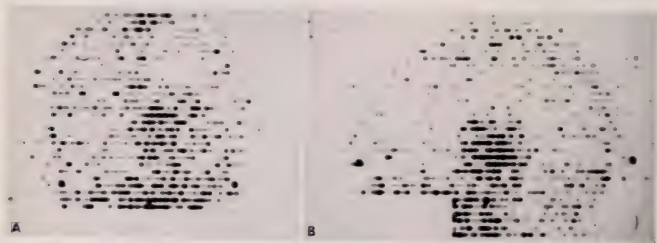


FIG. 6. 48 hour (A) anterior and (B) left lateral scans in a patient with an astrocytoma involving the left thalamic region. The scans were used for localization purposes at the time of needle biopsy. Angiography indicated a deep avascular lesion.

tions. Except where air actually outlines a lesion, pneumoencephalography may give only a relatively gross idea of its exact location.

An abnormal concentration of radioactivity as visualized on the brain scan indicates the actual site of an intracranial disease process. In cases where angiography demonstrates the presence of an avascular lesion, an abnormal scan showing a focal, well circumscribed lesion may be of exceptional localizing value, particularly if a needle biopsy through a burr hole is planned (Figs. 6A,B).

Occasionally, depending on the size and location of a particular lesion, brain scanning may be positive when angiography or pneumoencephalography are negative or indefinite. Ventriculography may be necessary to confirm an abnormality visualized on the brain scan in the case of midline lesions which obstruct the ventricular system (Figs. 7A,B).

#### LIMITATIONS OF BRAIN SCANNING

Although the abnormal brain scan almost always indicates the presence of a pathological process, the negative scan, on the other hand, does not

offer the same degree of assurance that disease is absent. Brain scanning is often ineffective in the small percentage of cases where low-grade gliomas or infiltrating types of neoplasms are present. In addition, certain intracranial mass lesions, depending on their size and location, are particularly liable to elude detection by brain scanning.

Lesions smaller than two to three centimeters in diameter are usually beyond the resolution of current instrumentation and often only the largest of many discrete metastatic foci may be detected. Posterior fossa lesions are frequently overlooked both because of their small size and the fact that they are difficult to distinguish from their rather considerable background of vascular and soft tissue radiation.

A similar situation contrives to make detection of lesions involving the floor of the cranial fossa equally difficult, even when these lesions are rela-

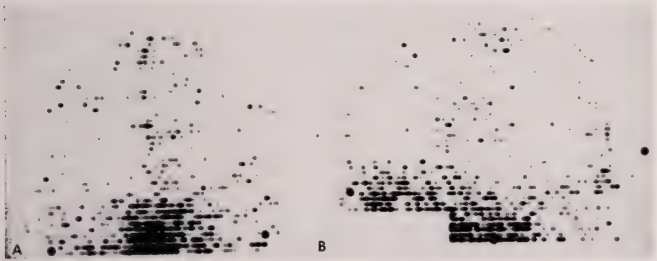


FIG. 7. 24 hour (A) anterior and (B) left lateral scans in a patient with a glioblastoma of the corpus callosum. The lesion was confirmed with ventriculography. Angiography was non-specific in this case.

tively large. For this reason, pituitary and para-sella tumors, as well as other types of extra-dural growths such as chordoma and craniopharyngioma are probably better visualized by other methods of examination.

Despite these minor limitations, the negative brain scan is still of considerable value and may play an important role in the management of the patient with suspected intracranial disease.

The major diagnostic limitation of the radioisotope examination is the current inability of this technique to delineate the nature of the pathological process it does detect. Any of a variety of intracranial conditions varying from the most benign to the most malignant types of disorders may make themselves manifest by reflecting an increased concentration of radioisotope.

Although there are sometimes striking differences in regard to the rate of uptake and order of concentration of tracer agents by different types of lesions, specific diagnosis solely by means of brain scanning with radioisotopes remains a controversial matter (2, 3, 5).

TABLE I

*Brain Scan Findings in 79 Cases with Histologically Verified Intracranial Neoplasms*

Histology	Number of Cases	Brain Scan	
		Positive	Negative
Astrocytoma	5	3	2
Chondrosarcoma	1	0	1
Ependymoma	1	1	0
Glioblastoma	17	16	1
Glioma, mixed	2	2	0
Glioma, unclassified	1	0	1
Lymphoma	1	0	1
Meningioma	23	21	2
Metastatic carcinoma	19	18	1
Metastatic melanoma	3	2	1
Pinealoma	1	0	1
Schwannoma (acoustic neuroma)	4	4	0
"Secondary" neoplasm	1	1	0
Total	79	68	11

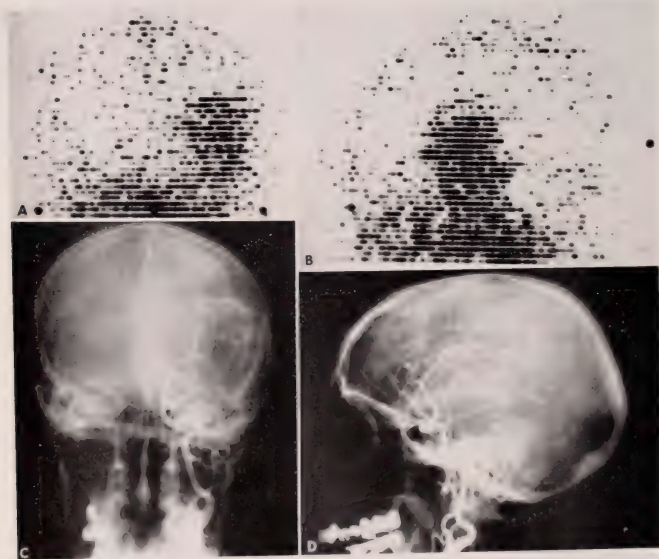


FIG. 8. 24 hour (A) anterior and (B) left lateral scans in a patient with a glioblastoma of the left temporal lobe. (C) and (D): corresponding angiographic views in the same case.

## RESULTS AND DISCUSSION

*Verified Cases*

To date, 84 patients of the 461 cases scanned have had intracranial lesions which were verified histologically. Scanning correctly localized lesions in 72 of the 84 verified cases. A number of pathological conditions were encountered. Seventy-five patients proved to have various types of neoplasms. Five patients had non-neoplastic mass lesions.

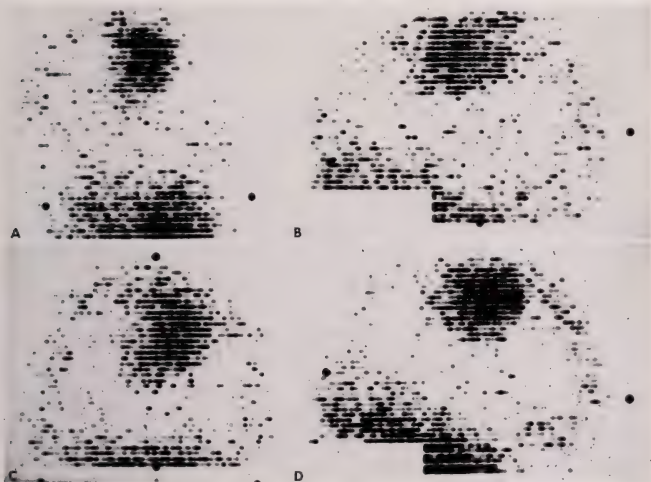


FIG. 9. Immediate (A & B) and delayed (C & D) anterior and left lateral scans in a patient with a recurrent falx meningioma.

*Neoplasms*

Table I illustrates the results of brain scanning in patients with verified intracranial neoplasms. Most neoplasms were detected on the delayed scan. As a group, meningiomas tended to appear on the immediate scans and to become more distinct on the delayed studies. Occasionally, this was true of other types of neoplasms as well (Figs. 8, 9, 10).

*Astrocytoma.* The two astrocytomas not detected by brain scanning proved to be infiltrating tumors. One lesion was in the region of the mid-brain. The other was a large, clinically indolent tumor involving almost the entire right hemisphere.

*Glioblastoma.* The single glioblastoma not detected was an infiltrating growth which diffusely involved the white matter of both sides of the brain.

*Glioma, unclassified.* This was another clinically indolent tumor with some of the histological characteristics of an oligodendroglioma.

*Meningioma.* All the supra-tentorial meningiomas in this group were correctly localized by scanning. The two undetected meningiomas were in the posterior fossa.

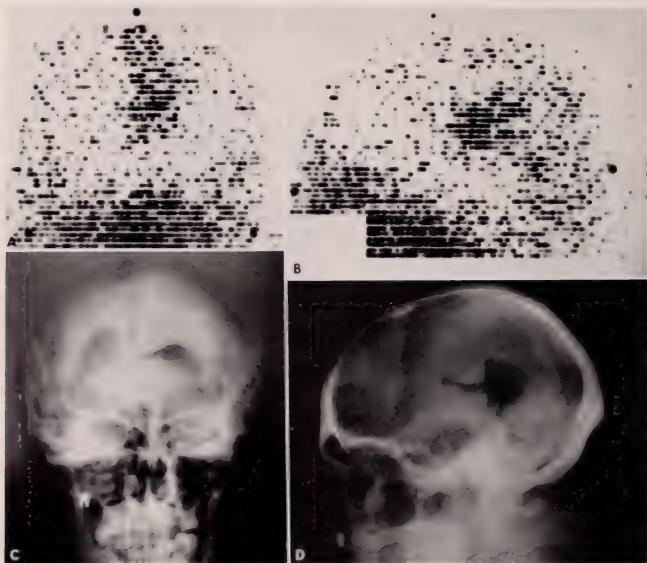


FIG. 10. 48 hour (A) anterior and (B) left lateral scans in a patient with a left mesial parietal metastatic deposit. (C) and (D): pneumoencephalogram in the same case.

*Metastatic tumors.* Metastatic deposits were correctly localized in 20 patients; however a number of these patients had more than one lesion. Usually, only the largest lesion was detected in such cases. Tumors under 2-3 cm in diameter ordinarily were not detected.

#### *Non-neoplastic Mass Lesions*

Table II illustrates the brain scan findings in five cases with verified non-neoplastic intracranial mass lesions. All the lesions diagnosed in this group were detected at 48 hours (Figs. 11A,B).



### Posterior Fossa Lesions

Table III reviews the results of brain scanning in the 12 patients with posterior fossa lesions. Results in this group were disappointing. On the other hand, all four schwannomas (acoustic neuromas) were correctly localized.

TABLE II

*Brain Scan Findings in 5 Cases with Histologically Verified Non-neoplastic Intracranial Mass Lesions*

Histology	Number of Cases	Brain Scan	
		Positive	Negative
Intracerebral hematoma.....	2	2	0
Subdural hematoma.....	1	1	0
Subdural hygroma.....	1	1	0
Tuberculoma.....	1	0	1
Total.....	5	4	1

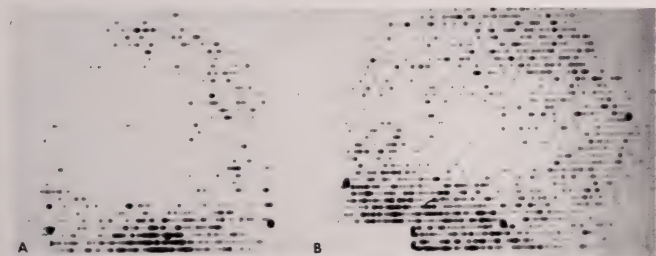


FIG. 11. 48 hour (A) anterior and (B) left lateral scans in a patient with a left sided subdural hematoma.

### Non-verified Cases

#### X-Ray Mass Lesions

Table IV reviews the results of brain scans in 69 cases where the diagnosis of an intracranial mass lesion was made on the basis of angiography or pneumoencephalography. The presumptive pathological diagnosis based on the x-ray findings or other clinical information is given where possible. A number of cases cannot be so categorized and are simply listed as having a "mass lesion." Some of the patients in this group have improved from both a clinical and radiological point of view. The other patients are still being followed for a more definitive diagnosis.



TABLE III

*Brain Scan Findings in the 12 Cases with Histologically Verified Lesions of the Posterior Fossa*

Histology	Number of Cases	Brain Scan	
		Positive	Negative
Astrocytoma	1	0	1
Ependymoma	1	1	0
Meningioma	2	0	2
Metastasis	1	1	0
Schwannoma (acoustic neuroma)	4	4	0
Lymphoma	1	0	1
"Secondary" neoplasm	1	1	0
Tuberculoma	1	0	1
Total	12	7	5

TABLE IV

*Brain Scan Findings in 69 Cases with X-Ray Evidence of Intracranial Mass Lesions*

Presumptive Diagnosis	Number of Cases	Brain Scan	
		Positive	Negative
Meningioma	4	2	2
Sella tumor	3	1	2
Angle tumor	4	2	2
Metastatic tumor	22	20	2
Mass lesion	31	17	14
Subdural	5	4	1
Total	69	46	23

TABLE V

*Brain Scan Findings in 29 Cases with X-Ray Evidence of Vascular Lesions*

X-Ray Diagnosis	Number of Cases	Brain Scan	
		Positive	Negative
AVM	6	5	1
Aneurysm	4	0	4
Internal carotid occlusion	9	3	6
Middle cerebral occlusion	4	3	1
Posterior cerebral occlusion	1	0	1
Small vessel occlusion	4	1	3
Arteriosclerosis	1	1	0
Total	29	13	16

*X-Ray Vascular Lesions*

Table V reviews the brain scan findings in 29 cases where lesions of the vascular tree were demonstrated by angiography.

*Arterio-venous Malformations.* Five of six patients with arterio-venous

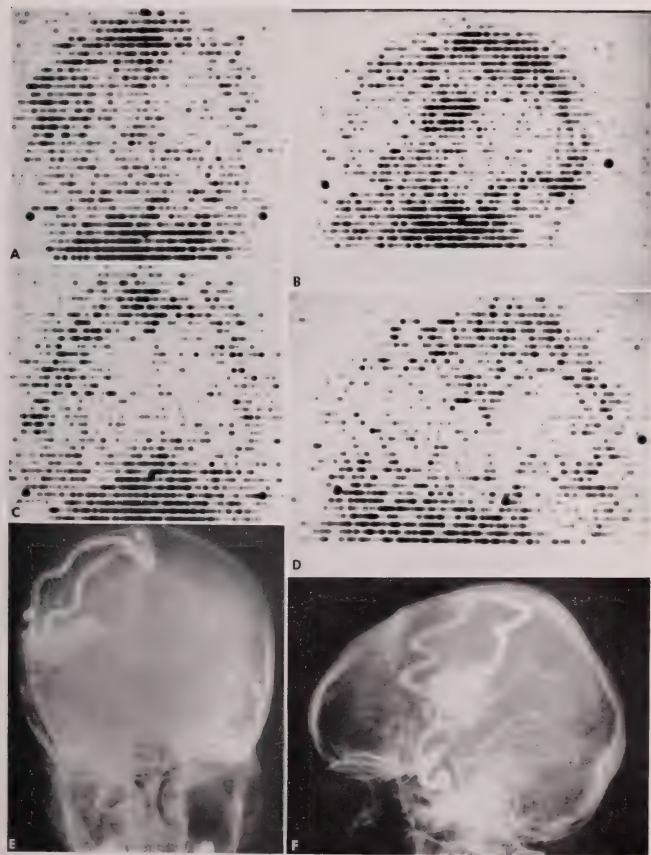


FIG. 12. Immediate (A & B) and 48 hour (C & D) anterior and right lateral scans in a patient with an arterio-venous malformation. (E) and (F): corresponding angiographic views in the same case.

malformations (AVM) were correctly diagnosed by brain scanning. The undetected lesion lay in the posterior fossa. These lesions were most striking on the immediate scans and tended to become less obvious on delayed studies (Figs. 12A-F).

*Occlusive Vascular Disease.* Patients with occlusions of the internal carotid artery were more likely to have abnormal scans during the acute phase of their



FIG. 13. 48 hour (A) anterior and (B) left lateral scans in a patient with an extracranial occlusion of the left internal carotid artery. Immediate scan was normal. (C) left carotid arteriogram in the same case. (A pneumoencephalogram revealed no evidence of a mass lesion.)

illness. Most patients with angiographic evidence of smaller vessel occlusions had negative scans. In general, the more profound the clinical picture, the more likely that the scan would be positive in this group. Repeated scanning of patients with angiographically proven occlusive vascular disease demonstrated that the abnormality visualized on the scan performed during the acute phase would disappear after a period of some weeks despite only slight clinical improvement on the part of the patient. The abnormal scans seen in cases of cerebrovascular disease were not dissimilar from the abnormal scans seen with neoplasms or other mass lesions in some cases (Figs. 13, 14).

### *X-Ray "Normals"*

180 of 188 patients examined by means of contrast studies and considered "normal" from an x-ray point of view also had "normal" or negative brain scans. Eight patients had abnormal radioisotope studies.

Two patients were clinically suspected of having cerebrovascular disease, but angiography failed to demonstrate evidence of occlusive phenomena. Repeat scans in both these patients were negative after an interval of some weeks. Clinical signs and symptoms also regressed during the same period.

Two patients had diffuse abnormalities on the side of a prior craniotomy.

The four remaining patients in this category still remain undiagnosed. Metastatic disease is suspected in two cases with proven carcinoma. In all four cases the lesion detected by brain scanning conformed to the site of the clinical localization of the patient's disease.

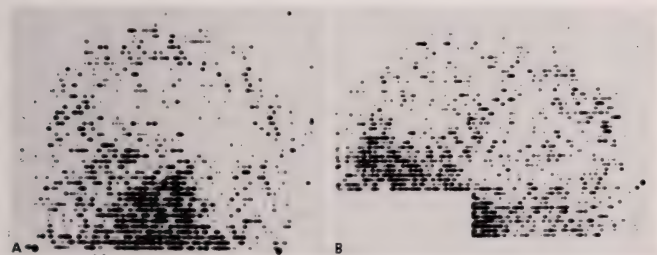


FIG. 14. 48 hour (A) anterior and (B) right lateral scans in a patient with a complete occlusion of the right middle cerebral artery. Immediate scan was normal.

### *Inconclusive Cases*

69 patients had no further examinations other than brain scanning. 22 patients had incomplete or unsatisfactory x-ray studies or scans, or inconclusive pathological findings.

Table VI summarizes the results of brain scanning in the 461 patients in this series.

### CONCLUSION

In the 84 verified cases in this series to date, the diagnostic accuracy of brain scanning with RISA was 86%.

Table VII illustrates the diagnostic accuracy obtained with a variety of tracer agents currently used for brain scanning. All the reports are based on verified material (1-4, 7).

Investigators reporting the highest levels of accuracy have restricted their series to include only intracerebral and meningeal neoplasms. The lower figures are based on the reports of investigators who have chosen to include all

types of intracranial lesions in their series. The overall results suggest that the choice of tracer agent does not influence the diagnostic accuracy of the radioisotope examination. Meningiomas, glioblastomas, and metastatic deposits have been universally detected with an extremely high degree of accuracy. Conversely, lesions at the base of the skull, cystic lesions, and

TABLE VI  
*Results of Brain Scanning in 461 Patients*

Case Material	Number of Cases	Scan	
		Positive	Negative
Verified Cases.....	84		
79 Neoplastic lesions .....		68	11
5 Non-neoplastic mass lesions .....		4	1
Non-Verified Cases.....	286		
69 X-ray mass lesions .....		46	23
29 X-ray vascular lesions.....		13	16
188 X-ray "normals".....		8	180
Inconclusive Cases.....	91		
69 Brain scans only.....			
22 Unsatisfactory studies.....			
Total.....	461		

TABLE VII  
*Accuracy of Diagnosis of Intracranial Lesions by Brain Scanning with Various Radioisotopes*

Investigator(s)	Year	Tracer Agent	Cases Verified	% Correct
Sweet and Brownell (7)	1955	As <sup>74</sup> (positron emitter)	131	76
Di Chiro (2)	1961	RISA (I <sup>131</sup> )	106	84
Feindel <i>et al.</i> (3)	1962	RISA (I <sup>131</sup> )	45	88
Feindel <i>et al.</i> (3)	1963	Radio neohydrin (Hg <sup>203</sup> )	64	88
McAfee and Fueger (4)	1964	RISA (I <sup>131</sup> )	141	71.5
Affi <i>et al.</i> (1)	1965	Radio neohydrin (Hg <sup>203</sup> )	62	84

low grade gliomas have not been detected with any appreciable accuracy regardless of the tracer agent employed. Most of these lesions are either beyond the resolution of current detection devices, obscured by superimposed background radioactivity, or simply do not take up sufficient concentrations of tracer agents to permit detection.

It is of interest to note that Moore originally pointed out that astrocytomas were the most frequently encountered nonfluorescent tumors in his fluorescein series (8).

The use of more sensitive detection devices and higher concentrations of

radioactivity derived from ultra-short lived radioisotopes may solve some of these problems. The development of new tracer substances with specific affinities for different pathological entities would be of even more importance.

Brain scanning with radioisotopes has proved to be effective in the detection and localization of a variety of intracranial lesions. The fact that brain scanning is a benign procedure suitable for out-patient screening and follow-up studies makes it of great value to the clinician involved in the diagnosis and management of intracranial disease.

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# Unusual Morphologic Anomalies of Chromosomes

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We wish to call attention to striking morphologic chromosome anomalies encountered in a patient with multiple somatic anomalies. In a previous communique (1) we considered the intriguing but remote possibility that thalidomine was the responsible agent.

T. was born on Cyprus in September, 1955. His father and mother, then thirty-one and thirty years of age respectively, and brothers, then eight and ten years of age, were all in excellent health. The family history was negative. Four months before becoming pregnant with T. G., the mother had a spontaneous sixth month abortion. There was no record of an examination of the fetus. Her pregnancy with T. was unremarkable and she was never exposed to significant amounts of x-ray. Specifically, she had had only infrequent diagnostic chest x-rays during her lifetime. In the summer of 1962, at the height of the thalidomine exposé, the parents wrote asserting that the mother was given physician's samples of Distaval† during the first trimester of the aborted pregnancy which she kept and consumed in the first trimester of her pregnancy with T.

Delivery was at term and was uncomplicated. Birth weight was 8 pounds, 6 ounces. The following somatic anomalies were noted: Enlarged head with occipital meningoencephalocele; hypertelorism; hemangioma of the midline face (2) including upper lip, nose and forehead; colobomata iridis and choroidea (3); short webbed neck; short bulky extremities; short index fingers; continuous palmar creases and undescended testes. X-rays showed changes in the proximal femoral epiphyses suggestive of dysplasia epiphysares punctata, a hypoplastic odontoid process and fusions between cervical vertebrae. His hemoglobin was usually in the range of 10.0 to 11.0 grams but most other laboratory data were normal. The occipital meningoencephalocele was removed shortly after birth and now, at eight years of age, he is grossly mentally retarded but ambulatory. He contracts frequent "colds" and ear infections.

Chromosomes were studied from the peripheral blood by the method of Moorhead *et al.* (4). Striking morphologic abnormalities were encountered: Elongation and thinning of arms of chromosomes was seen in at least one chromosome of 25% of cells. With seeming significant frequency, the affected chro-

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† Distillers Company Limited, London, brand of thalidomide.



mosome could be identified as a group A autosome. Often, however, affected chromosomes could not be satisfactorily classified. Additionally, isochromatic breaks were seen in at least one chromosome in 11% of cells and the arms of chromosomes were apparently running or sticking together in 27% of cells. These morphologic findings are based on the microscopic examination of fifty-six consecutive metaphase spreads. Twenty-four consecutive metaphase plates were then karyotyped and it was found that six or 25% had an abnormal complement

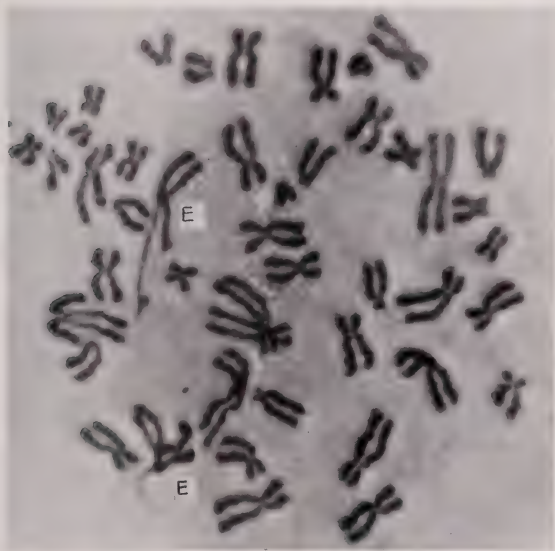


FIG. 1

of sex chromosomes. Thus, five cells were of an XXY constitution, one was XX and the remainder were XY. Of interest was the belated finding of a 5% occurrence of polymorphonuclear drumsticks, for the buccal smear was only 1% sex chromatin positive. A study of the bone marrow several months later revealed similar elongation and thinning anomalies.

There are a number of reasons to believe that these morphologic anomalies are not artefactual: 1. Morphologic changes of this sort had not been observed in two hundred cases previously studied at this laboratory. 2. Abnormal metaphases were often found lying beside normal ones. 3. Numerical sex chromosome anomalies were coexistent. 4. Examination of the bone

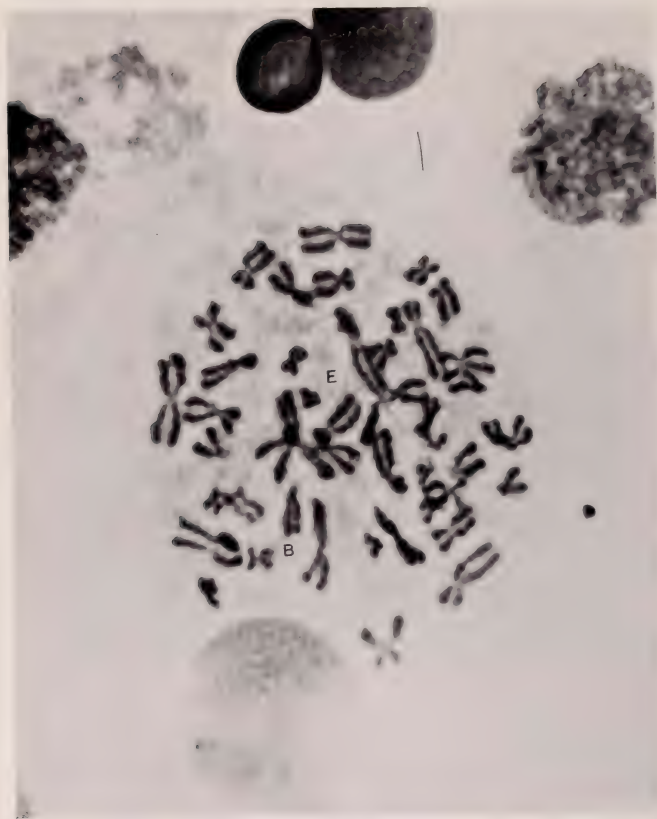


FIG. 2

marrow after an interval of several months showed identical anomalies. Neither was the phenotype incompatible with visible chromosome defects.

It was firmly contended that the mother consumed thalidomide early in pregnancy. Also, certain of the patient's anomalies suggested this cause.

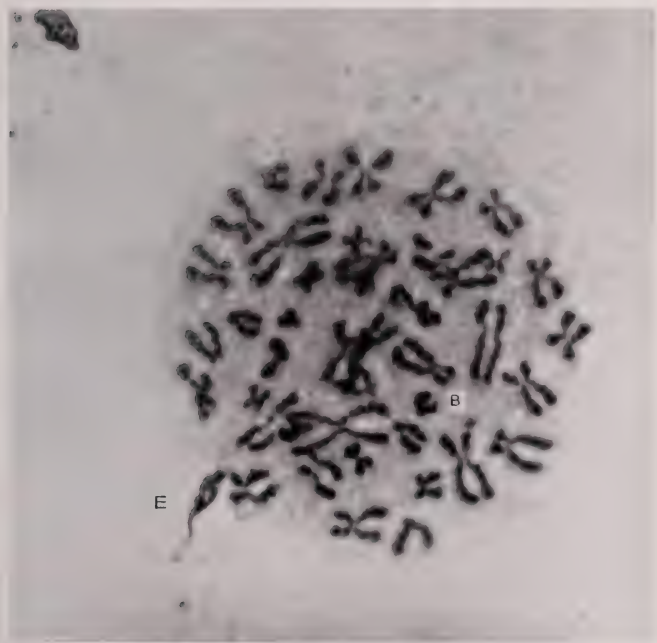


FIG. 3

However, the early date of the case, the atypical somatic anomalies and the previously unheard of chromosome anomalies (5) made of this only an intriguing but remote possibility. No other etiology was apparent. It would appear that patients with multiple, extreme somatic anomalies due to thalidomide (6) have not been studied cytogenetically. Aside from breaks and chromosome adhesiveness, we are unaware of the presently reported morphologic chromosome anomalies in neoplasia or irradiated tissues.

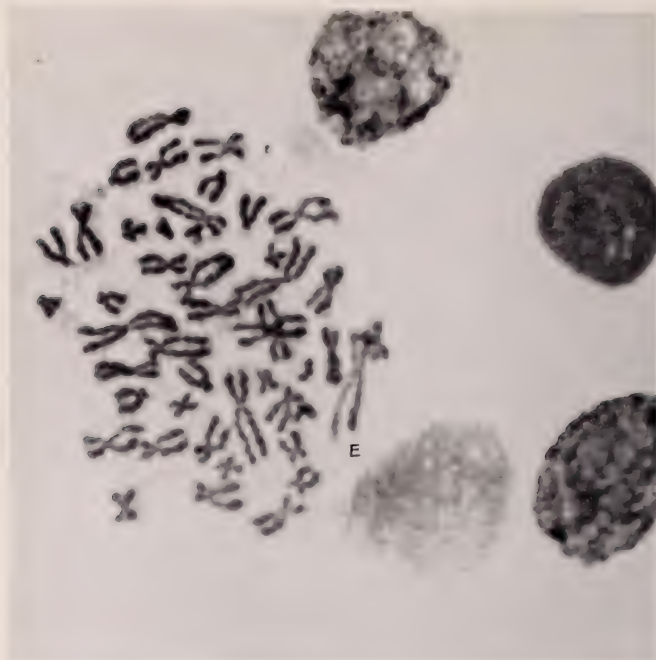


FIG. 4

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# The Adrenal Cortex and External Pancreatic Secretion in the Dog

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The effect of adrenocortical hormones on gastric secretion is well documented (1) but not at all clearly elucidated. Although the action of these hormones on pancreatic secretion has not been as fully studied, the variance in opinion of observers, both in human and experimental studies, is as great.

In man, Pfeffer and Hinton (2) studied the effects of hydrocortisone and ACTH in 50 patients subjected to the secretin test; and found that these hormones had no effect on bicarbonate or volume, but gave a low normal output of amylase. Dreiling, Janowitz and Rolbin (3) on the other hand, have demonstrated that ACTH and hydrocortisone statistically diminished the spontaneous flow, bicarbonate and amylase secretion in their subjects.

In experimental animals, two recent papers have attempted to delineate the effects of these hormones on pancreatic secretion. Sircus (4) studied dogs with chronic duodenal fistulae and found that cortisone and hydrocortisone given acutely, increased the concentration and output of bicarbonate in pancreatic secretion; whereas chronic cortisone administration gave a diminished bicarbonate response. In both acute and chronic experiments, cortisone and cortisol produced an increased output of amylase and trypsin. Nelp and co-workers (5) again studied the effects of cortisone on pancreatic function in chronic fistula dogs in response to secretin-pancreozymin and food, and found no change in amylase, bicarbonate or chloride concentration but a rise in volume output with secretin and pancreozymin. On the other hand, when a meal was used as the stimulus, there was an increase in flow rate and bicarbonate concentration, but a decrease in amylase.

We have recently described (6, 7), the determination of a maximal response of the dog pancreas to both secretin and pancreozymin. This method affords good reproducibility of results, and it was decided to apply maximal stimulation to studies of the effect of adrenocortical steroids on exocrine pancreatic function of the dog. As well as determining the effect of prolonged cortisone, and acute cortisol, ACTH, and aldosterone, the effects of adrenalectomy have also been studied.

## METHODS AND MATERIAL

8 female mongrel dogs weighing between 18 and 22 kg were prepared with chronic pancreatic fistulae as previously described (6), and were distributed as follows:

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1) 2 animals were standardized with maximal secretin and pancreozymin given as a single rapid intravenous injection. One dog was then given 50 mg of cortisone acetate orally daily for 70 days, and the other 25 mg cortisone acetate daily for 91 days. The response to a maximal test was assessed at two weekly intervals and the drug then rapidly discontinued. Further tests were then performed at weekly intervals to assess any change in function.

2) 3 animals were standardized with maximal and submaximal secretin given as a continuous infusion, and the effects of cortisol (hydrocortisone)\* 100 mg, ACTH 40 units,† and aldosterone‡ 7 micro-Gm/kg given as rapid intravenous injections 30 minutes prior to infusion of secretin were studied. The experiments were performed in tandem, the daily response to secretin being determined prior to the administration of the adrenal hormone.

3) 3 dogs were standardized with maximal secretin and pancreozymin given as a single rapid intravenous injection, and the effects of intravenous cortisol 100 mg, ACTH 40 units, and aldosterone 7 micro-Gm/kg 30 minutes before the second administration of secretin-pancreozymin in an in tandem assay of pancreatic secretion.

Bilateral total adrenalectomy was performed in 3 dogs which had been previously standardized. Two dogs were then studied with maximal secretin-pancreozymin as a single injection, and one animal with continuous infusion of secretin at maximal dosage. The animals were maintained on 2 Gm sodium chloride and on 0.5–1.0 mg DOCA per day, the DOCA being discontinued for 24 hours prior to each study. In the post-adrenalectomy state, the effect of intravenous cortisol, ACTH, and aldosterone was again determined in tandem studies, as was the effect of intravenous infusion of isotonic sodium chloride. After completion of these studies, maintenance therapy was discontinued and further studies done while the animals progressed to more complete adrenocortical insufficiency.

During the experiments, the procedure followed was as that previously described (6), the samples being collected in glass centrifuge tubes, sealed and immediately placed in ice. The bicarbonate concentration was estimated by the manometric Van Slyke method, the chloride with the Cotlove chloridometer, amylase by a modified Somogyi method and sodium and potassium with the flame photometer.

## RESULTS

1. *Response of the dog pancreas under maximal secretin and pancreozymin to the chronic administration of cortisone acetate.* Figure 1 shows the results in the animal given 50 mg cortisone daily. While on cortisone therapy, there is no significant change in the 15 minute volume, and bicarbonate output or the maximal bicarbonate concentration until 50 days on steroids, when there is a drop in volume. However there is a significant decrease in both the 15 minute

\* Hydrocortisone sodium succinate (Upjohn).

† ACTH (Parke, Davis & Co.).

‡ Aldosterone—d-aldosterone in 95% ethenol 1 mg/ml (Ciba).



amylase output and maximal amylase concentration, most marked after 50 days of therapy, but showing a return towards normal after 70 days on cortisone. When the cortisone was discontinued, the test at 10 days after cessation of therapy showed a marked drop in volume, bicarbonate output and concentration and amylase concentration and output. At 17 days after cessation, there is a trend towards recovery of pancreatic function, with all parameters

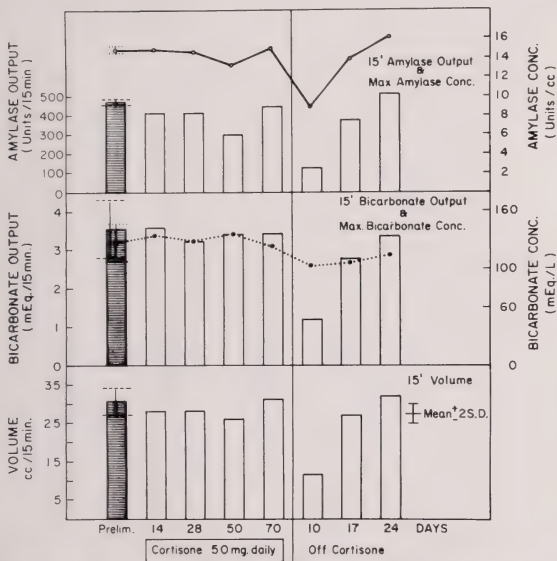


Fig. 1. Effect of 50 mg Cortisone Acetate daily on maximal pancreatic secretion in response to secretin and pancreozymin. Volume, bicarbonate concentration and bicarbonate output after cortisone are compared to precortisone levels  $\pm 2$  standard deviations.

back to pre-treatment levels 24 days after stopping steroids, except that the maximal amylase concentration is higher than the pre-cortisone values.

The pattern of response in the animal given 25 mg cortisone daily for 91 days was similar, the significant changes being a decrease in amylase output which is most marked after 91 days of cortisone. There was also a significant decrease in volume and bicarbonate output at 50 days on steroids. In the experiments performed at 14, 50, 70 and 91 days on steroids there was a significant increase in maximal bicarbonate concentration. When tested 3 days after cessation of the steroid, there was still a decrease in amylase output. However at 10 days after cessation, the amylase output had returned to normal values.



2. *Effect of I.V. cortisol, ACTH and aldosterone on pancreatic secretion in response to submaximal continuous infusion of secretin.* Figure 2 shows the results of these experiments. Cortisol 30 minutes before infusion of secretin had no statistically significant effect on volume, bicarbonate output or maximal bicarbonate concentration ( $p > .9$ ). ACTH also had no statistically significant effect on the three parameters studied ( $p > .5$ ). The effect of aldosterone was

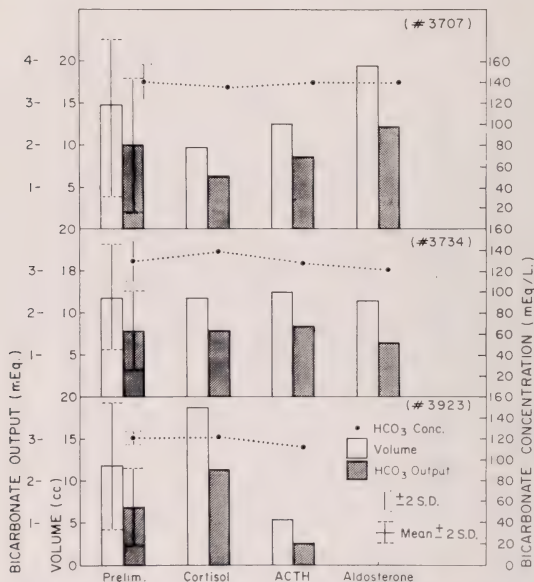


Fig. 2. Effect of intravenous cortisol (100 mg), ACTH (40 mg), and aldosterone (75 mg) on pancreatic secretion in response to submaximal constant infusion of secretin. The bicarbonate output and concentration responses in 3 animals are illustrated.

assessed in two dogs and again there was no significant difference with this hormone ( $p > .8$ ).

However when the results were assessed on the basis of tandem experiments, cortisol tended to give an increase in volume and bicarbonate output, ACTH tended towards a decrease in volume and bicarbonate output, and aldosterone produced an increase in these parameters in one animal, but no change in the other.

3. *Effect of I.V. cortisol, ACTH and aldosterone on pancreatic secretion in response to maximal continuous infusion of secretin.* In these experiments,

cortisol had no significant effect on the 15 minute volume, bicarbonate output or concentration in the three dogs ( $p > .5$ ), and ACTH gave an increased volume and bicarbonate output in one animal, but had no significant effect in the other dog ( $p > .2$ ). Aldosterone had no effect on these parameters in one dog, but gave a significant decrease in bicarbonate output and volume in the other animal ( $p < .05$ ).

When the results were again assessed on the basis of tandem experiments, ACTH and cortisol had no effect on maximal volume and bicarbonate; and aldosterone gave a decrease in one animal, and no change in the other.

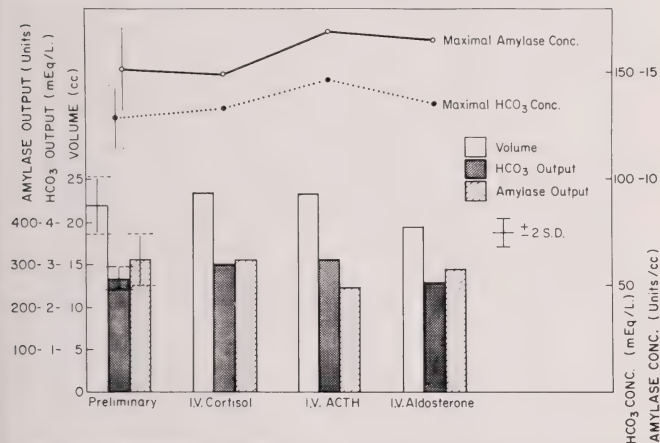


Fig. 3. Effect of intravenous cortisol (100 mg), ACTH (40 mg), and aldosterone (75 mg) on response of the dog pancreas to maximal single injection of secretin and pancreozymin (4 units/kg and 10 units/kg). Volume, bicarbonate concentration and output, and amylase concentration and amylase are illustrated before and after hormonal administration.

In all experiments, the sodium and potassium showed no change, and the chloride varied inversely with the bicarbonate. Amylase concentration was not estimated in experiments employing secretin alone.

4. *The effect of I.V. cortisol, ACTH and aldosterone on pancreatic secretion in response to a maximal single injection of secretin and pancreozymin.* Three dogs have been studied, and Figure 3 shows the results of these experiments in an illustrative dog. In response to cortisol, all 3 animals showed no change in 15 minute volume or amylase output, but two showed a rise in bicarbonate output. With ACTH, there was no change in volume, and one animal showed a rise in bicarbonate output but a fall in amylase output. In response to aldosterone, two animals showed no change but the third showed a significant decrease in volume, bicarbonate and amylase outputs.

When the results of all the experiments in all the dogs were analysed, maximally and submaximally stimulated pancreatic secretion was not changed to a statistically significant degree by cortisol, ACTH or aldosterone (Table I).

TABLE I

*Acute Effect of Cortisol, ACTH and Aldosterone on Submaximal Secretin-Stimulated Pancreatic Secretion. 3 Dogs Studied*

	Prelim.			Cortisol			ACTH			Aldosterone		
	No. Tests	Mean	SD	No. Tests	Mean	SD	No. Tests	Mean	SD	No. Tests	Mean	SD
Vol. (cc/15 min.)	10	13.0	6.02	3	13.4 $p > .9$	3.9	3	10.4 $p > .5$	3.7	2	15.5 $p > .8$	4.0
HCO <sub>3</sub> Conc. (mEq/L)	10	131	12.1	3	132 $p > 1.0$	7.3	3	127	11.5	2	130	10.0
HCO <sub>3</sub> output (mEq/15 min.)	10	1.69	0.88	3	1.71 $p > 1.0$	0.41	3	1.32 $p > .5$	0.58	2	1.87 $p > .5$	0.59

*Acute Effect of Cortisol, ACTH and Aldosterone on Maximal Secretin and Pancreozymin Stimulated Pancreatic Secretion. 6 Dogs Studied*

	Prelim.			Cortisol			ACTH			Aldosterone		
	No. Tests	Mean	SD	No. Tests	Mean	SD	No. Tests	Mean	SD	No. Tests	Mean	SD
Vol. (cc/15 min.)	30	22.7	6.9	6	20.9 $p > .5$	6.5	5	23.4 $p > .5$	4.6	5	17.5 $p > .2$	7.5
HCO <sub>3</sub> Conc. (mEq/L)	28	132	5.8	6	132	4.5	5	133	7.6	5	132	3.6
HCO <sub>3</sub> output (mEq/15 min.)	28	2.92	0.91	6	2.67 $p > .5$	0.80	5	2.99 $p > .9$	0.53	5	2.24 $p > .2$	1.15
Amylase concn. (Units/cc)	13	15.0	1.03	3	14.8	0.14	3	14.9	1.40	3	16.3	0.30
Amylase output (Units/15 min.)	13	335	100	3	363	91	3	325	78	3	333	177

#### THE EFFECT OF ADRENALECTOMY ON PANCREATIC SECRETION

Three animals were adrenalectomized and again subjected to maximal stimulation with secretin and pancreozymin. Tests were commenced eight days after adrenalectomy, and the animals studied for periods ranging from 30 to 50 days following adrenalectomy. Two animals were studied with maximal

secretin and pancreozymin given as a single injection. Figure 4 shows the results of 4 experiments in one of these animals post-adrenalectomy. In 3 experiments at 8, 22 and 29 days after adrenalectomy, there was a decreased 15

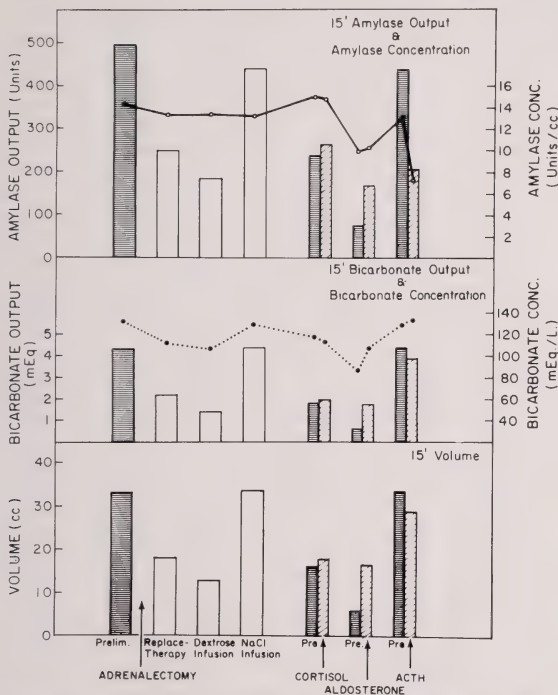


Fig. 4. Effect of bilateral adrenalectomy on maximal secretin and pancreozymin stimulated pancreatic secretion. The graph illustrates the effect of dextrose and saline infusion, cortisol, aldosterone, and ACTH on volume, bicarbonate and amylase output, and upon bicarbonate and amylase concentration in the post-adrenalectomy state.

minute volume, bicarbonate concentration and output, and amylase output ( $p < .02$ ). During these studies, the infusing fluid was 5% dextrose in water. Both cortisol and aldosterone, given intravenously, tended to increase these values but not to pre-adrenalectomy levels. In the fourth experiment, isotonic sodium chloride was infused during the test period and here the response to secretin and pancreozymin was normal in all respects. However the addition of

40 units of ACTH given intravenously, gave a decrease in all parameters except maximum bicarbonate concentration, the most marked effect being a diminished amylase concentration and output ( $p > .05$ ). At autopsy the pancreas weighed 50 Gm and was macroscopically and microscopically normal.

The second animal was well maintained on replacement therapy and in only one test, 15 days post-adrenalectomy, did she show a significantly diminished response to secretin and pancreozymin. The addition of cortisol, aldosterone or ACTH had no significant effect on any of the parameters studied, but it is noteworthy that the diminished response occurred whilst the infusing fluid was 5% dextrose in water.

When replacement therapy was discontinued, tests at 2, 5 and 9 days after cessation showed progressive decrease in volume, maximal bicarbonate concentration and output, and amylase output, but no change in amylase concentration. The administration of ACTH in these experiments caused an increased volume and bicarbonate output, a decreased bicarbonate concentration and little change in the amylase concentration or output. The animal suddenly died 12 days after cessation of maintenance therapy. At autopsy the pancreas weighed 31.7 Gm and looked normal. There was no evidence of any adrenal tissue.

The third animal was studied with continuous infusion of secretin at maximal rate (8 units min.), and a total of 11 studies were performed post adrenalectomy. Eight were done while the dog was on adequate replacement therapy. In only one of these experiments was the volume, bicarbonate output or concentration significantly diminished, and this occurred with infusion of 5% dextrose in water at 30 days post adrenalectomy. Cortisol, aldosterone and sodium chloride did not significantly alter the response to secretin in these studies, but ACTH produced a significant fall in volume, bicarbonate output and concentration. It is also noticeable that the studies during which sodium chloride was the infusion, showed higher values for all parameters than those in which dextrose in water was infused.

Maintenance therapy was then withheld, and further studies performed 5, 9 and 12 days after cessation of therapy. These tests showed a progressive decrease in volume, bicarbonate concentration and bicarbonate output, with the greatest fall occurring during infusion of 5% dextrose in water instead of sodium chloride. ACTH administration then caused a significant fall in all parameters, whereas cortisol and aldosterone caused a relatively small decrease. The sodium concentration of the secretin showed a progressive fall during the post-adrenalectomy period, and the potassium concentration a slight rise. The serum sodium levels also progressively fell and were lowest just prior to death of the animal, three weeks after cessation of replacement therapy. At autopsy the animal had obviously lost weight, but the pancreas felt and looked macroscopically normal, with its weight being 32 Gm.

#### DISCUSSION

Interest in the effects of corticosteroids on the pancreas was stimulated by the study of Carone and Liebow (8) on post mortem evidence of acute pan-

creatitis or peri-pancreatic fat necrosis in 16 of 54 patients treated with ACTH or various steroids. This was a histological diagnosis only, since none of these subjects showed symptoms of pancreatitis. Stumpf and co-workers (9) induced nonspecific acinar changes in 44 of 53 cortisone treated rabbits. These alterations were very similar to lesions described in rats and dogs after ethionine administration, and in experimental pancreatitis produced by other means. Sporadic case reports of pancreatic necrosis following cortisone therapy have appeared in the literature (10), and prompted studies of the effects of steroids on pancreatic secretion. However the wide variation in observations have left unanswered the nature of the physiologic effects of steroids on the exocrine pancreas. We have attempted to resolve some of these problems but have only been partially successful.

When cortisone acetate was chronically administered to our animals, certain clear-cut changes became evident. Cortisone therapy up to 91 days produced a significant decrease in the amylase output, but little change in volume or bicarbonate output. However in the animal given 25 mg cortisone daily, there was also an increase in the maximum bicarbonate concentration during the period of administration. These changes had all returned to pre-treatment levels from 2-4 weeks after cessation of the steroid, but at the same time there was noted an increase in amylase concentration and output. The animal given 50 mg cortisone daily showed a marked decrease in all parameters soon after cessation of the steroid. We believe that these findings indicate that the dog was in temporary adrenal insufficiency at this time since subsequent studies showed complete recovery of pancreatic function. Our results diverge from those of Sireus, who found an increased amylase output on cortisone. However he was working with submaximal stimulation and the two studies are not strictly comparable.

The effect of cortisol, aldosterone and ACTH was less clear cut. Acute cortisol, given with submaximal stimulation, showed a trend to increase volume, bicarbonate output and concentration but not to a statistically significant degree. It had no effect on maximally stimulated secretion. We believe that this suggests a potentiating effect of cortisol on pancreatic secretion, which is not obvious under conditions of maximal stimulation.

The effects of ACTH were more variable, but the trend was to decrease volume, bicarbonate concentration and output, and amylase concentration and output.

The response to aldosterone, on the other hand, was very variable, in some cases giving an increase in volume and bicarbonate, in other cases giving a decrease in these parameters. However again the trend for this hormone was to produce a decrease in the above parameters. From these studies, we can conclude that cortisol, ACTH and aldosterone given acutely produce no significant effect on pancreatic secretion.

Because of this variability, we adrenalectomized three animals in order to study the effect of these hormones in the absence of endogenous adrenocortical secretion, and to also study the effect of adrenal insufficiency on pancreatic secretion. Baker (11) had shown that adrenal insufficiency interfered with



secretory processes in the digestive tract, as indicated by a reduction in weight of the gastrointestinal mucosa, and reduction in gastric secretion, which was only partially corrected by DOCA and cortisone.

With the animals on adequate replacement therapy, cortisol and aldosterone tended to produce no significant changes in pancreatic secretion, but ACTH produced a fall in volume, bicarbonate and amylase activity in two of the three dogs studied. Adrenocortical insufficiency produced a significant fall in volume secreted, bicarbonate concentration and output, and amylase concentration and output. This was only partially corrected by cortisol and aldosterone, but isotonic sodium chloride infusion corrected the deficiency in volume and bicarbonate. Again in a state of deficient secretion, ACTH produced a further fall in all these parameters in two of the three animals studied.

These findings indicate a profound effect of adrenocortical insufficiency on exocrine pancreatic secretion. That this may be mediated through the effect of adrenocorticoids on blood volume and electrolyte composition was suggested by the partial correction of this defect following infusion of isotonic sodium chloride or the administration of aldosterone. These changes are interesting in view of the recent observations (12, 13) of the occurrence of steatorrhea in cases of Addison's disease. The steatorrhea is believed to be based on a small intestinal mucosal defect, but our findings indicate a possible lack of pancreatic enzymes as well.

It is difficult to explain the fall in volume, bicarbonate and amylase which occurred in the post-adrenalectomy state following the administration of ACTH. However it is apparent that the presence of functioning adrenal cortex is not a prerequisite for ACTH to exert its effects on the exocrine pancreas. The only feasible explanation is that commercial ACTH contains a substance or substances which have a specific action on the exocrine pancreatic secretion; and that this action is inapparent in the presence of normal adrenocortical function. Engel (14) has recently discussed the extra-adrenal actions of ACTH where he lists proven and possible extracortical actions. However there is no notation of a decreased pancreatic response under the influence of ACTH. The further elucidation of this action will have to await a better understanding of some of these extra-adrenal effects of ACTH.

#### SUMMARY

1. Eight female mongrel dogs with chronic pancreatic fistulae have been studied with maximal secretin and pancreozymin, and submaximal secretin, to determine the action of cortisone, hydrocortisone, aldosterone and ACTH on exocrine pancreatic secretion. Three animals were subsequently adrenalectomized and also studied with secretin and pancreozymin.

2. Prolonged cortisone administration caused a fall in amylase concentration and output in both animals studied, a rise in bicarbonate concentration in one animal, but little change in volume secreted.

3. Acute IV cortisol, ACTH or aldosterone gave no statistically significant changes with maximal or submaximal secretin and pancreozymin.



4. Adrenal insufficiency caused a profound fall in volume of juice secreted, in bicarbonate concentration and output, and in amylase concentration and output. This was partially corrected by intravenous aldosterone and by infusion with isotonic sodium chloride. ACTH in the post-adrenalectomy state caused a further decrease in all parameters studied whereas cortisol had no effect in these animals.

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# Prolonged Survival After Respiratory Insufficiency with Papilledema

## (A Case Report and Review of the Literature)

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### INTRODUCTION

Papilledema as a complication of chronic pulmonary disease has been frequently reported in the past two decades, and its pathophysiology has been elucidated. Long considered a grave prognostic sign, it has recently been emphasized that, by halting the progression of the underlying pulmonary disease, patients with papilledema may survive for a long period of time (1-3). We have had the opportunity of following such a patient for over six years since an episode of respiratory acidosis with papilledema.

As recovery from acute infection in chronic pulmonary disease is so often possible today, and more patients therefore reach an endstage of severe respiratory insufficiency, papilledema is seen more frequently in lung disease and an appreciation of its changed prognostic significance is of importance.

### CASE REPORT

This 74 year old white retired Army regular was admitted to the Coral Gables Veterans Administration Hospital on February 5, 1958, with somnolence present for several hours, cyanosis and emesis for several days, and fever and cough productive of rusty yellow sputum for two weeks. He had smoked four packs of cigarettes and consumed a case of beer or quart of whiskey each day of his adult life.

On admission physical examination, he was noted to be acutely and chronically ill, obese, dyspneic, cyanotic, confused and retching. Pulse rate was 108 per min, temperature 102.4°F. There was papilledema with engorged and tortuous retinal veins. Examination of the chest showed increased anteroposterior diameter, poor excursion, markedly decreased breath sounds and fremitus, and coarse râles and rhonchi. Heart tones were distant, the liver was enlarged and nontender and the epigastrium was tender.

Admission hemoglobin was 17.5 Gm%, hematocrit 63%, serum sodium 132 mEq/L, potassium 2.7 mEq/L, chloride 76 mEq/L and bicarbonate (venous) 44 mEq/L. Tests of liver function were normal. Chest roentgenogram revealed linear and nodular infiltrates bilaterally. Electrocardiogram indicated right bundle branch block, and right atrial hypertrophy. Sputum culture grew *Pseudomonas*.

Treatment with bronchodilators, alevaire, antibiotics, intermittent positive

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pressure and a cofflator was instituted. The patient vomited coffee ground material, became abusive and agitated. On February seventh, tracheostomy, and on February eighth, phlebotomy were performed. By February tenth, sensorium had cleared and cyanosis decreased. Progressive improvement continued. The tracheostomy was corked on February 14 and closed on February 28. On March 3, venous bicarbonate was 25 mEq L. Follow-up chest x-ray showed generalized radiolucency, widened intercostal spaces and slight reticular fibrosis, but clearing of the previous infiltrates. At discharge on March 12, the patient had no neurologic symptoms and few respiratory symptoms.

He has been followed at the U. S. Public Health Service Out-patient Clinic in Miami since his discharge, on a regimen of postural drainage, breathing exercises, prophylactic antibiotics, bronchodilators, bronchodetergents, potassium iodide and intermittent positive pressure breathing. Except for a period in 1959 of increased dyspnea and cough associated with lethargy and a rise in the venous  $\text{CO}_2$  combining power to 84.3 volumes per cent, for which therapy was intensified and corticosteroids and phlebotomy briefly employed, he has done well. He has moderate dyspnea on exertion, and slight cough productive of scant sputum. He is alert and moderately active. On physical examination, he has not changed significantly since discharge in 1958. The optic discs are pink and sharp, the retinal veins slightly distended and tortuous. Electrocardiograms no longer show right atrial hypertrophy, and the heart remains normal on roentgenographic examination.

(This is an elderly man with a typical history of obstructive pulmonary disease with infection. Since an episode of respiratory acidosis with cor pulmonale, neurologic changes and papilledema in early 1958, he has remained stable and independent and required no further hospital care.)

#### DISCUSSION

Stevens, Austen and Knowles (1), on their follow-up of three previously reported cases of respiratory insufficiency with papilledema (4), noted the patients to be living six years later. Two cases had required rehospitalization with acute respiratory insufficiency, cor pulmonale and papilledema, which had again improved. The authors reviewed twenty cases of papilledema associated with chronic pulmonary disease previously reported in the literature. Only nine had follow-up data. Of these, five died within five months of onset of papilledema, and the remaining four within two years.

Review of fifteen additional cases of chronic respiratory insufficiency with papilledema (Table II) revealed that seven died within 24 months of the first appearance of papilledema, three recovered during the initial hospitalization but had no further follow-up and four were still living but followed only three to twenty-nine months. Only one very recent case previously reported by this author (14) had a prolonged survival with adequate follow-up. He died five years after his first episode of papilledema in a course marked by progressive impairment and repeated hospitalizations (15).

Papilledema occurs in chronic lung disease as a consequence of cerebral

vasodilatation and increased cerebral blood flow resulting primarily from hypercapnia (16-20). It is therefore a manifestation of severe and longstanding respiratory insufficiency almost invariably associated with other neurologic changes and cor pulmonale. The development of physiologically sound concepts and means of treatment, particularly antibiotics, positive pressure respirators and tracheostomy, has made possible cessation of the progression of the underlying pulmonary disease, and prolonged survival following the appearance of papilledema. Papilledema may therefore not indicate a rapidly fatal outcome, and may be compatible with prolonged survival. This is con-

TABLE I  
*Pulmonary Function Studies*

Test	Observed	Per Cent of Predicted
Maximum Voluntary Ventilation	30 L/min	30%
Maximum Voluntary Ventilation after Isuprel Inhalation	37 L/min	37%
Vital Capacity (Forced)	1640 cc	47%
Vital Capacity (Slow)	3130 cc	90%
Residual Volume	3720 cc	240%
Total Lung Capacity	6850 cc	137%
	Observed	Normal
Timed Vital Capacities		
1 sec. per cent of total	27%	75%
2 sec. per cent of total	50%	85%
3 sec. per cent of total	62%	95%
R.V./T.L.C. Ratio	54.3%	Less than 35%
Helium Equilibration	4 min	Less than 2 min
Arterial Blood Gases, Resting, Room Air		
Oxygen Saturation	89%	More than 95%
pH	7.40	7.38-7.42
Carbon Dioxide Tension	57 mm Hg	38-45 mm Hg

sistent with the findings of Munck *et al.* (21) that the clinical picture and blood gas abnormalities were of less significance in prognosis than the pulmonary reserve of the patient before his acute illness.

#### SUMMARY

A case of chronic obstructive pulmonary disease has been followed for over six years without need for further hospitalization since an episode of acute decompensation with papilledema. Papilledema has always been considered a serious prognostic sign in chronic pulmonary disease, and prolonged survival after its occurrence has been reported only very recently. Improvements in understanding of pathophysiologic mechanisms and availability of physiologically sound methods of management have made possible halting the pro-

TABLE II

*Follow-Up of Cases of Papilledema Occurring in Respiratory Insufficiency*

Authors	Year	Case	Follow-Up after First Appearance of Papilledema
Friedfeld and Fishberg (5)	1934	1	Clearing of papilledema with therapy; no FU
Simpson (6)	1954	1	Death after two years
		2	Death during initial hospital admission
Sieker and Hickam (7)	1956	1	Recovery during initial hospital admission; no FU
Leggat (8)	1958	1	Still living at time of report 13 mos later
Miller, Bastron and Kearns (9)	1960	1	Still living at time of report 29 mos later
		2	Improvement during initial hospital admission without clearing of papilledema; no FU
		3	Death after an unspecified interval in a progressively downhill course
Manfredi <i>et al.</i> (10)	1961	1	Improvement; followed only three mos
Meyer <i>et al.</i> (11)	1961	1	Death during initial hospital admission
Crispin and Darke (12)	1962	1	Death after six mos
		2	Death after 14 mos
Imari (13)	1962	1	Death after 23 mos
		2	Still living at time of report 19 mos later, but bedridden because of loss of vision
Miller, Bader and Bader (14)	1962	1	Death after five years with repeated episodes of decompensation (15)

FU = Follow-Up.

gression of the underlying pulmonary disease and changed the prognostic significance of this sign.

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# **Ligamentum Denticulatum**

## **(An Anatomical Review and Its Role in Various Neurosurgical Problems of the Spinal Cord)**

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The dentate ligament, a ribbon-like structure, minute in size but significant in various pathological conditions of the spinal cord, has been infrequently written about in the last sixty years. In his book, *Tumors of the Spinal Cord*, Elsberg (1) observed its relation to the symptomatology of ventrally located intraspinal tumors. Kahn (2) related its role in myelopathy caused by cervical spondylosis and emphasized its section in decompression of the spinal cord. In vascular anomalies of the spinal cord, symptomatic relief has been observed after the section of the dentate ligaments (3). In 1961 (4) the myelographic visualization of the dentate ligament both in normal and pathological conditions of the spinal canal has been described.

After more than one-half a century, it seems worthwhile a brief review on the dentate ligaments with a particular reference to its role in various neurosurgical problems including, 1) extramedullary lesions such as (a) cervical or thoracic intervertebral disc protrusion, especially of the central type, (b) cervical spondylosis with compression of the spinal cord, (c) extramedullary neoplasm; 2) intramedullary lesions, such as (a) intramedullary glioma, (b) syringomyelia, (c) vascular anomalies of the spinal cord; 3) cordotomy.

### ANATOMY OF THE DENTATE LIGAMENT

The dentate ligament is a thin and narrow fibrous band constituted of longitudinally arranged fibers, situated on either side of the spinal cord. Its inner border is continuous with the pia mater and its lateral border laced with multiple digits. It begins at the top of the cervical cord and ends along the side of the conus medullaris. The cervical portion of the ligament is wider and thicker than the thoracic and lumbar portion. In the cervical area, the medial portion of the ligament may be very thin and easily severed from its pial attachment.

There are 21 digits of the dentate ligament. Each digit is triangular in shape, except the first and the last, and occasionally the eighth. The base of the triangular digit is in continuation of the main ligament, and its apex is stoutly attached to the inner surface of the dura. The first digit is more or less round and cord-like, attached to the dura mater opposite the margin of the foramen magnum, between the vertebral artery and the hypoglossal nerve. The last one breaks into a two-pronged fork which is 1 to 2 cm in length and may be as long as 3 to 4 cm. The lateral prong is the actual digit, and the medial prong

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is the tapering end of the dentate ribbon but sometimes it skips the side of the conus with only its lower end connected to the pia mater. The eighth digit is occasionally fork-like with short prongs.

The 21 digits are evenly distributed along the whole length of the spinal cord. Minus the first and the last, they are exactly the number of the cervical and thoracic vertebrae. The point of dural attachment of the dentate digit is in between the two adjacent traversing nerve roots and divides the spinal canal into a posterior and an anterior compartment, the former slightly larger than

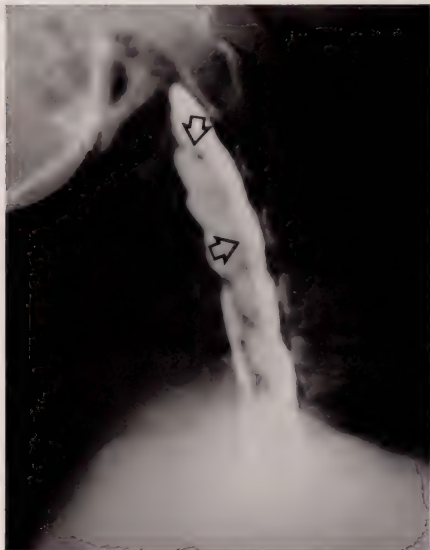


FIG. 1. Cervical Pantopaque myelograph. Arrows indicate dentate ligament.

the latter. It separates also the posterior from the anterior nerve roots, providing a landmark for their identification at surgery. The L1 sensory roots lie upon the last fork, which may be used as a landmark for their identification (5).

The spinal cord is suspended in the spinal canal by the dentate ligaments and to a lesser extent by the nerve roots. The dura to which the dentate is attached, is adherent to the anterior wall of the spinal canal. The lateral attachment and vertical arrangement of the dentate ligaments allow very little rostro-caudal and lateral movement of the spinal cord, however, it permits the spinal cord some degree of motion in the anterior posterior direction. Thus,

section of 2 to 3 dentate digits on each side would increase the anteroposterior and lateral mobility of the spinal cord to gain access to the anterior subarachnoid space and the anterior spinal canal.

*Myelographic identification of the dentate ligament.* In the lateral and oblique projection of a "full-column" Pantopaque myelograph, the dentate ligament can be visualized as a linear radiolucent shadow. Because of its greater thickness, the cervical portion of the ligament is more readily visualized

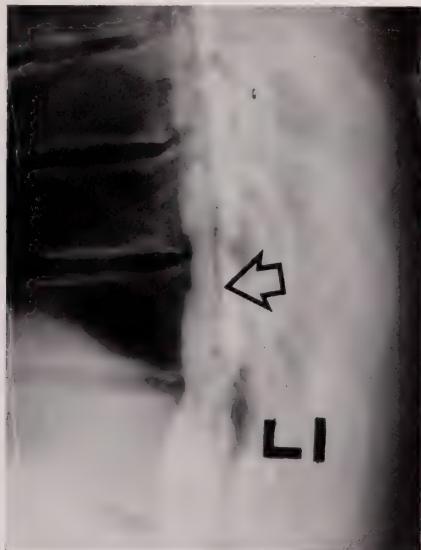


FIG. 2. Lower dorsal Pantopaque myelograph. Arrow indicates dentate ligament.

than the dorsal section (Figs. 1, 2). In cervical spondylosis, the myelographic continuity of the dentate ligament may be disrupted or distorted (4). The dentate ligament may be displaced by an intraspinal neoplasm, either extra-medullary or intramedullary. The direction of its displacement is indicative of the location of the tumor in the ventral or posterior spinal canal (Fig. 3) (Fig. 5, Case 1).

#### THE ROLE OF DENTATE LIGAMENT IN CORD COMPRESSION

Kahn (2) in collaboration with Zweig pointed out from the mechanical (stress) point of view, that in anterior spinal cord compression caused by a

central disc or osteophytic ridge, on account of the fixation of the cord by the dentate ligament, the main intramedullary stress was projected in the posterior lateral fasisculus dorsal to the ligamental attachment. The author presented it as one explanation of the pyramidal manifestation in anterior cord compression.

In the same token, in posterior compression of the spinal cord by an extramedullary mass lesion would place the stress anterior to the dentate ligament in the anterior lateral fasisculus. This may affect the spinothalamic tract, producing hypalgesia or paresthesia, a variety of subjective sensory complaints such as tingling, burning, numb or cold feeling below the level of the lesion, which Elsberg (1) described in his book of spinal cord tumors, often led to the misinterpretation as an anteriolaterally situated lesion. In the latter location,



FIG. 3. Dorsal Pantopaque myelograph in a case of meningioma of the posterior spinal canal. Large arrow indicates depressed dentate ligament. Small arrows outline the tumor.

the tumor was wedged between the dentate slip and the dura, and the anterior lateral surface of the cord. The sensory change was probably caused by direct compression of the anterior lateral fasisculus.

Myelographic evidence of dentate displacement has been also observed in intramedullary tumors (Case 1, Fig. 5) and syringomyelia. The direction of dentate displacement was opposite to the bulk of the tumor or cyst. The distortion of the dentate digits, therefore, may produce intramedullary stress in addition to the pressure caused by the expanding neoplasm.

In vascular anomalies of the spinal cord, the loss of medullary tissues and gliosis may increase the tension of the dentate digits and produce stress across the spinal cord (Fig. 4), as one observes the effect by pulling upon two opposite edges of a piece of paper. This may provide a hypothetical explanation to some of the symptoms of paresthesias and pyramidal involvement in vascular anomalies of the spinal cord, in spite of the fact that pathology was mainly confined

in the posterior portion of the spinal cord. At surgery the tension of the dentate digits has been demonstrated (3).

#### THE ROLE OF DENTATE LIGAMENT IN SURGERY OF THE SPINAL CORD

Since the dentate ligaments divide the subarachnoid space into an anterior and a posterior compartment, for the approach to the anterior spinal canal, it is imperative to mobilize the cord without traction by section of the dentate attachments. The cord is incapable to sustain traction, and unduly traction accounts for postoperative morbidities in many instances of centrally ruptured cervical intervertebral disc and thoracic disc. In such cases a transdural re-

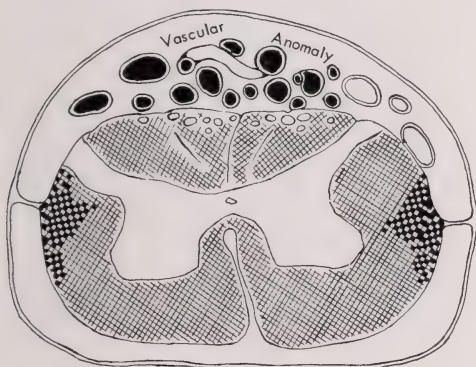


FIG. 4. Diagram of vascular anomalies of the spinal cord. Areas of stress in the lateral fasciculus (checker-board).

moval of the disc after the section of the dentate digits is safer than the extradural approach. One of the 5 cases thus treated is presented here (Case 3).

The thoracic spinal canal is narrow and deep which renders the exposure of the anterior spinal canal more difficult than the cervical region. For the removal of a ruptured dorsal intervertebral disc, or a ventral intradural or extradural tumor, adequate exposure of these lesions could be accomplished after the section of 2 to 3 dentate digits and 1 or 2 thoracic nerve roots. The portion of the spinal cord can be easily mobilized by rotation without traction. Three cases of ruptured thoracic intervertebral disc and 4 cases of ventrally located neurofibromas, including 3 intradural and one epidural, were successfully treated by the transdural route.

The section of the dentate ligament as one type of surgical treatment of cervical spondylosis with compression of the spinal cord has been frequently described in recent literature and further discussion is omitted from this presentation.

In cordotomy, the dentate ligament has been used as an anatomical indicator to guide the incision. Occasionally the ligament might be found detached from the lateral surface of the spinal cord, and in such instances, a careful search would reveal a slightly thickened pial line which could be accurately secured with a mosquito clamp to direct the cord incision. I have found in no instance to use the anterior roots as a land-mark in cordotomy.

#### ILLUSTRATIVE CASES

Whenever there is displacement of the dentate ligament, there is intramedullary stress produced by increased tension of the dentate ligament. Therefore, in compression of the spinal cord, there is a dual force of pressure, 1) the pressure exerted upon the cord by the extramedullary or intramedullary tumor, and 2) intramedullary stress concomitantly occurred as a result of dentate displacement. Thereby, the section of the dentate ligaments of the compressed area may procure an additional effect of decompression, an internal decompression in addition to the external decompression effected by laminectomy.

##### *I. Decompression in Intramedullary Tumor*

*Case 1.* A 36 year old school teacher was admitted to the hospital on April 15, 1957 on account of marked weakness in all 4 limbs for 2 days and painful stiffness of his neck for about 2 years. Since 1951, he has been treated at other clinics as a case of "multiple sclerosis."

Examination showed only feeble extension and flexion of the left elbow and fingers of his left hand. The right upper extremity was completely paralyzed. He was unable to move his right lower limb but weakly raised his left leg 30 degrees off bed. He was unable to move his toes of either foot. Hypertonicity was obvious in all limbs. The upper extremities were held in flexion and both lowers in extension. Atrophy of the interossei was obvious in both hands. Anesthesia was demonstrated on the left from below the clavicle, and hypalgesia on the right. Vibration and position sense were lost on both sides. He was urinary incontinent. His respiratory movements were largely diaphragmatic. He was mentally confused and disoriented in time and space. Ankle and wrist clonus, Hoffmann's and Babinski's sign were demonstrated on both sides. Tenderness was elicited over the spinous process of the entire cervical segments.

A spinal block was demonstrated in CSF manometry. The initial pressure of the CSF was 218 mm of water and the total protein content was 144 mg%.

A cervical Pantopaque myelogram showed a fusiform enlargement of the cord from C2 to C4. The lateral projection showed that the dentate ligament was displaced ventrally (Fig. 5).

On April 19, 1957, laminectomy was performed from C2 to C5. The cord was noted markedly enlarged and the dentate attachments were found stretched downward. Three small vertical incisions were made into the cord and an inoperable intramedullary hemangioma was disclosed. The tumor extended from C2 to C4, and its surface resembled that of a ripe mulberry (Fig. 6). The stretched dentate attachments from C2 to C6 were sectioned on both sides. The dura was left open and tented with Vinyon "N" cloth.

Postoperatively, he showed a rapid returning of pain, temperature and touch sensation of the right side of his body and the right lower extremity. Three days later, he was able to make a weak grip with his left hand and raise his left arm above his head, and move his right fingers. Normal chest movements were noted in respiration. He was able to move his toes of both feet. On the fifth day pain sensation appeared in the left arm and leg, and the left side of his body. Spastic contractions of extremities had disappeared. The relief of pain and spasm of the neck, shoulders and arms was complete.



Radiotherapy was initiated on the 18th postoperative day, and a total of 2,400 r (air units) was given. Four months later, he was able to sit in a wheel-chair, feed and dress himself.

The patient was last examined on February 25, 1964. He could write and type with his left hand. The left hand grip was strong and the right weak. Hypertonicity was demonstrable in all 4 limbs, right more than the left. Pain, temperature and touch sensation were normal in the right side and a vague sensory level of hypalgesia was elicited from below D7 on the left. Position and vibration sense remained absent on both sides.

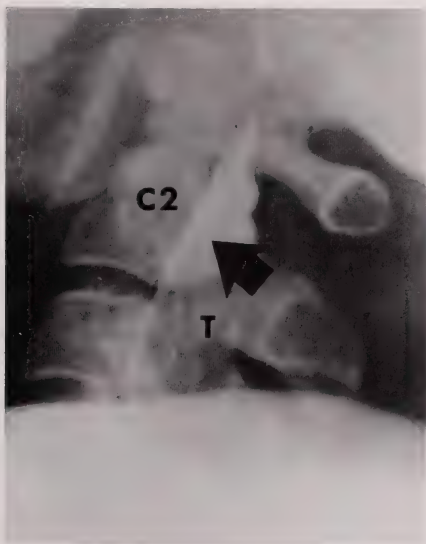


FIG. 5. Case 1, Cervical Pantopaque myelograph. Arrow indicates displaced dentate ligament. T—indicates site of an intramedullary hemangioma.

Pathologic reflexes were present in all 4 extremities. He was able to walk on crutches. The bladder control remained lost.

*Comment:* In this case, an extramedullary decompression was accomplished by laminectomy, and a probable intramedullary decompression by the section of the dentate ligaments. The displacement of the dentate ligaments by the tumor was obvious on the myelography, and the section of the dentate attachments has probably contributed partially to the effect of cord decompression observed in the early weeks following the operation. The effect of irradiation was doubtful and the small myelotomies were by no means adequate in decompression.

Two cases of cervical intramedullary glioma and one case of syringomyelia

have been similarly treated without myelotomy, and the follow-up period was 36, 28 and 46 months respectively. To date, all 3 patients are still fully employed, and there has been no noticeable increase in neurological defects. In fact, improvement has been observed in all 3 patients in the early postoperative weeks.

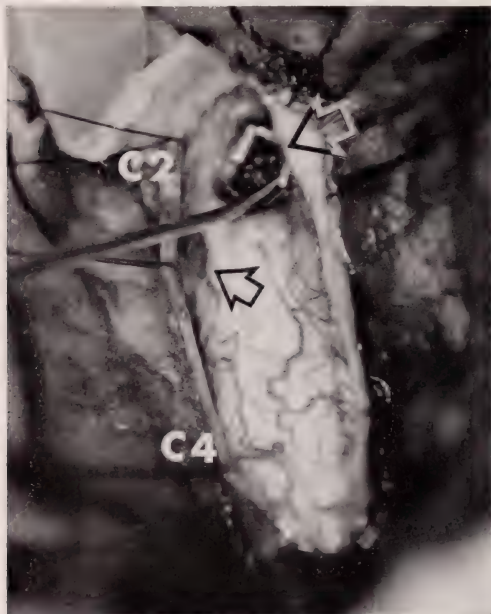


FIG. 6. Case 1, operative findings. Note the tumor exposed by myelotomy (upper arrow).

## *II. Section of Dentate Ligaments in Vascular Anomalies of the Spinal Cord*

*Case 2.* A 54 year old man complained of burning paresthesias and weakness in both lower extremities for 8 months. Both legs felt heavy and he was unable to walk more than two city blocks.

Neurological examination disclosed mild weakness in both lower limbs. Hypalgesia was found shifting from D7 to D9 at different examinations. Vibration sense was impaired from below D7 on both sides. There was bilateral Babinski's sign.

Radiograph of the spine was noncontributory. The CSF manometry demonstrated a slow rise and fall. The total protein content was 93 mg%. A Pantopaque myelography showed tortuous vascular shadows extending from D3 to D11.

On June 28, 1962 laminectomy from D3 to D11 was performed and disclosed an extensive venous anomaly covering the dorsal surface of the spinal cord (Fig. 7). The dentate

attachment from D3 to D11 was sectioned on both sides. The dura was left open and tented with Teflon cloth.

Following the operation the relief of burning pain in both lower limbs was complete, and he was able to walk ten blocks. Vibration sense remained lost in both lower extremities. The level of hypalgesia was not demonstrable. Babinski's sign was still

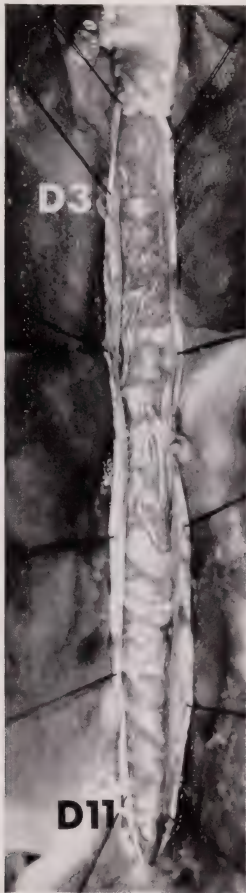


FIG. 7. Case 2, Venous anomaly from D3 to D11.

obvious. His neurologic status remained unchanged 14 months after surgery. There was no urinary difficulty.

*Comment:* Decompression by laminectomy alone in the treatment of vascular anomalies of the spinal cord has been known ineffective. The relief of



FIG. 8. Case 3, C5-C6 ruptured disc.

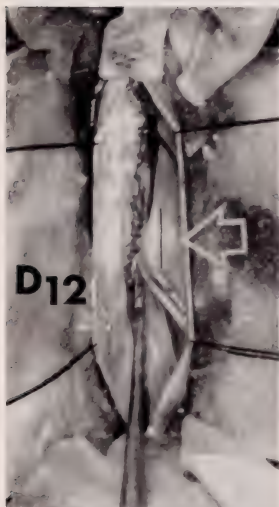


FIG. 9. A case of ruptured D11-12 intervertebral disc. The spinal cord was mobilized to the left without traction after the section of the dentate ligaments, and the ruptured disc was fully exposed (arrow). Black line indicates dural incision for the disc removal.

symptoms in this case may be attributed to the section of the dentate ligaments by intramedullary decompression.

The origin of pain and paresthesia, which are prominent symptoms in vascular anomalies of the spinal cord, was probably in the lateral spinothalamic tract. Section of the dentate ligaments has been performed in 15 patients and the follow-up period varied from 2½ to 7 years. A satisfactory relief of pain was observed in 7 patients and partial relief was noted in 3. Receding of sen-

sory level was observed in 5 and improvement in motor function of the extremities was found in 6 patients. Three patients showed no improvement following surgery.

### *III. Section of Dentate Ligaments in Approach to the Ventral Spinal Canal*

*Case 3.* A 50 year old man had pain in the back of his neck and both shoulders for 4 months. On Sept. 25, 1962, during a cervical manipulation by a chiropractor, he suddenly dropped to the floor and became immediately quadriplegic.

On arrival at the emergency room, examination showed no voluntary movement in any of his 4 limbs. The upper extremities were flaccid and the lower spastic. A sensory level of anesthesia was demonstrated at D3. The respiration was abdominal. The upper limbs were areflexic and the lowers were hyperreflexic with bilateral Babinski's sign, ankle and patellar clonus. The bladder was distended with 1,200 cc. of urine.

The CSF manometry demonstrated a complete block which was visualized at C5-6 in a Pantopaque myelograph.

A laminectomy was performed at C5 and C6. Two dentate digits were sectioned on each side and the cord was mobilized to the right without traction. The ventral dura was opened and a large completely ruptured central disc was removed (Fig. 8).

Next day he regained his bladder function which was demonstrated both by the residual urine test and by cystometry. The sensory level receded to D7 and gradually disappeared in a week. He was able to light his own cigarettes and feed himself on the fourth postoperative day. One month later, he walked with a cane which was discarded after 4 months, but his gait was spastic.

*Comment:* In addition to this case, another 4 patients of ruptured central cervical intervertebral disc, and 3 patients of thoracic intervertebral disc have been operated upon by the transdural route after a liberal section of the dentate ligaments (Fig. 9). Improvement has been observed in all 8 patients. Seven patients were fully employed and one committed suicide 18 months after surgery.

A ventral thoracic epidural neurofibroma had been removed transdurally from a 13 year-old paraplegic boy with complete recovery. Again, it is to be emphasized that a liberal section of the dentate ligaments with the sacrifice of one or 2 thoracic nerve roots could provide adequate exposure to the ventral spinal canal without traction on the spinal cord. Furthermore, the section of the dentate ligaments may ease pressure caused by edema of the cord either as a result of trauma or surgery.

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# Psychiatric Aftercare Services: Their Place in the Continuum of Patient Care

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The term aftercare suggests a process that follows a period of treatment of an illness usually extensive, of a serious nature and more often than not one confined to a hospital stay. The word "care" in aftercare has the same root as the word cure which connotes an action and state of healing. It is also synonymous with concern, solicitude, anxiety and worry—states normally present in the person who cares to cure. Both care and cure are implied in the word treatment which is more commonly used in the art and science of healing.

The word "after" in aftercare implies that a state of treatment and care existed before, which perhaps was more important than the treatment of the aftercare period, perhaps even life-saving. This same element is also contained in another expression often used to designate aftercare, namely follow-up. My association to the word follow-up is a mopping-up or cleaning-up action, after a major intervention or operation. Follow-up in medicine implies somewhat of a standby situation destined more to watchfulness than action. Aftercare is an active process rather than a passive state of watching and observing. We prefer the term aftercare rather than follow-up. Psychiatry has for too long been associated with passivity in the minds of people because of a paucity of available remedies. Since this is no longer so, we are justified in using the more active term of aftercare.

The question as to whether aftercare in psychiatry is less important or less active than the care or treatment that preceded it is still an open one. Thus far a distinction has been made between care defined as main treatment requiring full-time care, more often on an inpatient basis, and aftercare defined as collateral care and treatment, on a part-time basis and usually in the out-patient department clinic. I cannot resist the temptation to bring in another member of the care family, namely forecare. Although we are usually humble when it comes to considering preventive psychiatry, and rightly so, and although effective prevention of mental illness may be some time away, I believe that forecare is clearly related to care and aftercare, and that one of the avenues that may lead to effective prevention will stem from effective care and aftercare on family and community levels. Care on these levels is bound to have a beneficial effect not only on the patient but on the family and others as well.

Having distinguished between aftercare, care and forecare I hasten to add that the distinction is artificial, that aftercare and forecare can be nothing

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*Presented at the Monthly Departmental Conference, October 14, 1964, by the staff of the Aftercare Clinics of the Institute of Psychiatry: Samuel L. Safirstein, M.D., Chief, Peter Laqueur, M.D., Maurice Osinoff, M.D., Bertrand Jacobs, M.D., and Edmund Slakter, M.D.*



else than care as a multifaceted problem, and that any division or subdivision can only be considered for heuristic purposes, while the whole of the problem as well as the whole of the individual must be constantly kept in mind.

The purpose of this paper is to highlight what is done and what is going on in the Aftercare Clinics of The Mount Sinai Hospital, Department of Psychiatry, and to point out some of the immediate and long-range problems as well as to introduce a discussion of a philosophy of aftercare treatment, and to envisage possible implications of an aftercare concept.

Shortly after the Institute of Psychiatry was opened in December of 1962 it became apparent that the Aftercare Clinic was on the threshold of important changes. They stemmed from two primary sources. The number of inpatient beds increased fourfold (this does not include the Day and Night Center) and the number of residents increased fivefold. As the adult inpatient units began to discharge their expanded numbers of patients, at the beginning of 1963, wheels began to turn faster and faster at the Aftercare Clinic. The numbers of

TABLE I  
*Patient Population of Psychiatric Aftercare Clinic 1959-1964*

	1959	1960	1961	1962	1963	1964
Census January 1	42	53	60	58	77	178
New Admissions	45	36	32	46	152	211
Readmissions	13	19	11	19	31	23
Total Admissions	58	55	43	64	183	234
Cases Closed	47	48	45	45	82	140

On January 1, 1965 the census of patients in the clinics was 272.

patients began to rise sharply and our facilities and capacities became over-taxed. When the new contingent of residents arrived in July 1963 it became clear that we did not have enough physical space to contain the patient population and their therapists. We were blessed with numbers, we were growing and multiplying by leaps and bounds and we needed more room. Like a stock that went up in price we split two for one. We divided into two parts, Clinic A which remained in the Klingenstein Clinical Center and Clinic B which moved across the street to Carver House. Four members of the Attending Staff supervise these clinics and act as Preceptors to the house staff. Table I illustrates the numerical changes in the aftercare population before and after the opening of the Institute of Psychiatry.

We see from this data that the relatively small number of 152 admissions to the OPD Aftercare Clinic in 1963 reflects the fact that it took some time before all the four adult inpatient units were in full operation. The figure 211 for 1964 is the new admissions rate that can be expected. This is the number around which the saturation point will be reached. Together with readmissions we can expect a total annual admission rate to the Aftercare Clinics of about 235 patients. This figure slightly exceeds the average of 54 patients per unit

that we had on Ward A from 1959 through 1962, before the Institute opened. I think it reflects a more rapid turnover of patients on the wards. We also note that the readmission rate only increased slightly despite the fourfold increase in the patient population. The rate of patients closed also increased, as shown by the figure 140 for 1964.

At the beginning of 1965 we had 94 more patients than at the beginning of 1964 and at the beginning of 1964 we had 101 more patients than at the beginning of 1963. This indicates an increase of about 100 patients annually in the residual caseload of the clinics. I fear to project the census of the Aftercare Clinics in 1975 if we proceed at the same admission and closing rates.

If there is one thing certain about the future of the Aftercare Clinics it is that they will continue to grow numerically. What we hope is that they grow in an arithmetic and not geometric proportion. The structure and modus operandi of the Aftercare Clinics (1) is predicated upon the basic unit of the resident and patient involved in a therapeutic relationship. The patient is admitted immediately following discharge from the ward and is cared for by the same resident who was his therapist on the inpatient unit. The transition from treatment on the ward to the clinic is abrupt, as therapy time is reduced to one-half hour weekly. An additional half-hour session is allowed when necessary and the resident has time available. The resident's supervisory time is also sharply reduced to one-half hour weekly for his entire patient load. Every new admission is interviewed by the supervisor usually in the presence of the resident therapist; following the interview, supervisor and therapist determine goals of and plans for aftercare treatment. As the patient finds his place in his home and in the community, his sessions at the clinic are spaced and eventually discontinued. If his condition becomes aggravated and rehospitalization is advisable, he may be returned to The Mount Sinai Hospital inpatient service or sent to city or state facilities for continued care.

In the basic resident-patient relationship two main processes take place concomitantly, the treatment of the patient and the training of the resident (2). These two processes are kept in mind by the supervisor during the weekly sessions with the resident. Beside this basic individual psychotherapy unit, a number of other services are available in the Aftercare Clinics.

Certain patients not amenable to intensive psychotherapy but receiving drugs are transferred to a special unit called the Medication Clinic. This unit is not only instrumental in keeping the patient on medication, but also evaluates, modifies, reduces and eventually discontinues medication. Modification, reduction, and discontinuance of medication are important functions not only in the Medication Clinic but throughout individual therapy by residents.

The inpatient services rely heavily on medication, in the all-out assault on the patient's acute phase of illness, and almost every patient who is admitted to the clinic is on medication. It is a fundamental function of the clinics to aim at discontinuance of medication, when indicated, in preparation for discharging

the patient. It has been our experience that once medication is relied upon, the tendency of the therapist is to "forget" to reduce the dosage, to continue psychotherapy only to realize quite late that the patient's dosage is too heavy and he now resists attempts at reducing it.

The rationale for using drugs is not questioned here. What is questioned is the length of time patients are maintained on drugs in the clinic and the lack of attempt to reduce the dosages. It is very difficult to discharge a patient who is on drugs.

The Medication Clinic alone now has 30 patients, many of them are known as chronic patients in other clinics of the hospital and usually belong to a low socio-economic group. They cling to Mount Sinai. This residual group of patients has a low rehabilitation and discharge rate.

Since 1955 group therapy has been one of the modalities of treatment employed by the Aftercare Clinics. Currently we have two groups of 20 patients engaged in a supportive type of psychotherapy. We follow the principle that group therapy is not an adjunct to individual therapy but a different kind of therapy with its own techniques, goals and dynamics for which candidates have to be specially selected. Once a patient is in group therapy he does not receive individual psychotherapy, except in emergency situations. We would like to expand our group therapy program. We think that one purpose for which groups might be utilized is to effect a smoother transition toward discharge from the clinic. It seems that the problems of separation can be better handled in a group where the transference is diluted.

An important working relationship is maintained between the Aftercare Clinics and the Social Service Department. Psychiatric casework with significant relatives begun while the patient is on the ward, continues while he is in the clinic. The resident and caseworker continue as a team throughout the patient's stay in the clinic. Rare is the situation when a patient is not considered in the light of the caseworker's knowledge of the family constellation and the family's dynamics. In discussing difficult or emergency situations, the clinic chief may see the therapist, the caseworker and their respective supervisors. Several levels of knowledge and experience concerning the patient are in this way combined to determine a course of action.

The caseworker is an important member at the weekly conferences with the director of the Department of Psychiatry, at which time casework material is presented along with the therapist's material. We also frequently utilize the caseworker's knowledge of community resources when direct help is needed for the patient himself. I am referring here to such situations as contacts with school, the Department of Welfare, the Department of Rehabilitation, the housing authorities, job resources and the like.

It is my belief that there is room for even greater utilization of social service. For instance, when a patient is readmitted to the clinic, the casework contact is seldom reopened because of a shortage of caseworkers. Greater use of caseworkers could also be made in certain selected cases where direct casework with the patient would be the treatment of choice.

Hitherto the clinic relied primarily on the psychological work-up of patients done while they were on the inpatient services. The Department of Psychology is now able to absorb additional referrals, coming directly from the Aftercare Clinics. We intend to take advantage of this opportunity to retest selected patients, who have been in treatment for one year or longer, in order to compare possible changes due to treatment.

From even a brief consideration of Table I, it is apparent that certain measures are necessary to take care of the constantly increasing numbers of patients in the Aftercare Clinics. This makes it imperative to discuss a philosophy of aftercare treatment. Since it is impossible to consider aftercare without considering care, any discussion of aftercare will have to touch on inpatient care as well.

When I picture schematically inpatient care I see a man in a white coat with his front toward the hospital and his back toward the community. When I picture outpatient care I see a man with his back to the hospital and his front to the outside community. When we put the two men back to back, we have a care unit with alternating emphasis on inpatient or outpatient care.

The aftercare patient is weak and unsteady on his feet. He is helped to either regain his premorbid place in the community or is rehabilitated to occupy a somewhat different place. In either case aftercare is with him until he can function by himself. Even after his discharge his ties with the hospital and the clinic continue invisibly in his knowledge that should he require help in the future the doors of the clinic are open to him. This is what we tell patients when we prepare them for discharge. This is what keeps many of them going and willing to try life on their own. The "open door" policy in our aftercare clinic is a basic principle that carries important implications. One such implication is that unless the patient must be rehospitalized as a matter of emergency, he will come to the Aftercare Clinic first, whether it is a month, a year or 10 years after his discharge. It is the Aftercare Clinic that has the opportunity to evaluate his condition and determine a course of action which very often consists of readmission to the clinic for a brief period of supportive psychotherapy. Some years ago I presented such a case (13) which involved a 33 year old schizophrenic man, a former patient of the Aftercare Clinic who, following certain special circumstances, developed an acute anxiety attack and returned for help. With the information of his previous ward and clinic records, psychological and social service work-ups, this man was able to be discharged after 9 half-hour psychotherapy sessions and we have not heard from him since. Such brief readmissions to the clinic are common occurrences.

The "open door" policy can also be paraphrased as "once an aftercare patient, always an aftercare patient," which means that we have the opportunity to follow our patient not only in sickness but also in health and to intervene when the very first symptoms of recurring illness appear. It is in this sense that the aftercare clinic performs a preventive function with

its former patients, by maintaining invisible ties of unresolved transference with them and seeing them before there has been a complete break in their psychic equilibrium. The Aftercare Clinic is the first agency to which its former patients turn when in need. In a way it can be said that these patients are never discharged. They are merely taken off the active list.

If we agree then that the patient can reach us when in need, and if the patient and his relatives are aware of this, could we not try a faster discharge rate from the clinic? By using somewhat different criteria having to do with degree of functioning in the community, we could perhaps be more daring and encourage earlier discharges of our patients. We have been doing this on a small scale and on a trial basis in 1964 and this is reflected in the relatively high number of discharges (140) during that year.

The use of the words "health and illness" in reference to aftercare patients, in or out of the clinic, require definition in this context. I see mental illness as a chronic disease process acquired early in childhood which eventually becomes a way of life. There is structure and superstructure of drives and defenses against them on multiple levels of the personality make-up which are of a conflicting nature, setting up potentially eruptive and disintegrative patterns. These are kept in balance by homeostatic forces operative in every living organism. When homeostatic forces are at work, the individual is in a state of relative well-being, his anxiety level is low and he is often capable of a high level of functioning. For the purpose of this discussion as well as a practical approach to restorative psychotherapy, I call this state health. When homeostatic forces are overcome by disruptive conflict, anxiety becomes overwhelming and disease, disequilibrium and disintegration become pervasive. We have a state of decompensation. The first and foremost task of therapy is to create and foster circumstances that allow reintegration and homeostasis to be operative again. Successful treatment consists of restoring the patient to the state of functioning of which he was capable prior to becoming sick. What I call health then, is a chronic state of sickness in which the individual is able to function and derive certain satisfactions and pleasures from living. What I call sickness is an acute state superimposed on the chronic structure which brings about disintegration of the personality and dysfunction. I do not close my eyes to the chronic state of mental illness present in many of those capable of successful functioning but this belongs to the realm of reconstructive therapy (4). It seems to me that in a psychiatric unit of a general hospital we are first and foremost committed to restoration of function, a therapeutic aim followed by all medical disciplines.

Does this mean that all we are interested in is taking care of emergency situations and quieting down acute anxiety states? I should not feel uneasy if this were the only therapeutic goal, in certain psychiatric settings, for certain types of patients. There is enough challenge in this relatively modest therapeutic aim to keep many generations of psychiatrists busy. However, this does not have to be the only therapeutic aim. Each acute disequilib-



rium, disintegration and break of homeostasis offers the opportunity for reintegration on a slightly different basis than the one that existed before. If we can exercise our therapeutic influence, during the very sensitive period of reintegration, on a tiny segment or sector of the personality we can make a tremendous difference in the patient's future life. A small correction in a sensitive area of the patient's personality may have reverberating results in areas that really matter to the patient in his way of life. To mention a very brief clinical example, a 38 year old depressed, married woman with 2 children was treated on the ward without improvement. Decision for ECT was made and nine treatments were given. There was still no improvement, but before more ECT was decided upon the resident-therapist was able to reach his patient on the "real object" level. In the ensuing excellent doctor-patient relationship, the patient was permitted and even encouraged to ventilate her enormous anger toward her husband. After she left the ward and came to the Aftercare Clinic, she began to express her anger directly to her husband with whom she had been rather compliant in the past. A difficult period of actual marital battling followed, during which the patient found support in her therapist. Eventually the patient won important concessions in the way her husband treated her which permitted a satisfactory relationship and a good deal of satisfaction in her married life. She is now discharged. Each resident has one or more cases like this with whom meaningful, goal-directed and goal-limited psychotherapy is done.

I have heard the question asked: where is psychotherapy to be done? On the inpatient service or in the Aftercare Clinic? There are those who believe that all the wards should be concerned with, is to bring about compensation and reintegration and only when the patient comes to the clinic does meaningful psychotherapy begin and continue. Others, at the opposite extreme, believe that the only place where important psychotherapy can take place is on the inpatient service, while the clinic must concern itself with consolidation of acquired gains. I find the ward-clinic dichotomy artificial, arbitrary and actually harmful to our therapeutic endeavors. As I indicated before, wards and clinics are involved in the same process of care and psychotherapy is begun on the ward and continued in the clinic. The therapist, in the person of the resident, remains the same on the ward and in the clinic and at least for a period, assures such continuity. Later on, the Aftercare Clinic, the Department of Psychiatry and the hospital remain the symbol of continuity, not only for the patient but also his family.

I would like to make a case for more co-operative effort between the wards and the Aftercare Clinics. It seems that a live concept of ward-aftercare clinic continuum could be of benefit to both patient care and resident training. Here are some points that come to mind.

1. A clear condensed view of the patient upon his discharge from the ward is needed for his continuing care in the Aftercare Clinic. This could be contained in a short note indicating (a) goals of treatment on the ward, the



extent to which they were accomplished, modified, or abandoned, and (b) directions to follow concerning the future treatment of the patient according to the ward team. These are matters usually discussed at the discharge conference. The knowledge of the salient points of this conference would assist the Aftercare Clinic in its treatment plans. In the ideal situation a person from the Aftercare Clinic should be present at the discharge conference if at all possible.

2. It is generally agreed that the transition from ward to clinic is abrupt. As a consequence, worsening in the patient's condition in the first week after discharge from the ward is a fairly common phenomenon. At times the patient has to be rehospitalized. I wonder whether a weaning process prior to discharge from the ward might not reduce the possibility of early relapse. What I mean by weaning is a gradual reduction of individual psychotherapy sessions, discussion with the patient of the aftercare program and even introduction of the patient to the clinic's locale and its personnel.

3. Since all our patients come from the inpatient service, ward disposition of patients affects the Aftercare Clinics. Consideration might be given as to whether every patient is in need of aftercare treatment, as it seems that every patient discharged from the unit automatically becomes an aftercare patient. We even receive some private patients. Among those who do need aftercare treatment some might be followed in clinics closer to the patient's home; we have patients who travel considerable distances to come to the clinic. Thought could also be given to aftercare by private non-psychiatric physicians in certain selected, uncomplicated situations.

4. When it comes to research, mostly of a clinical nature, there is a tremendous opportunity and a wealth of material in the Aftercare Clinics awaiting interested individuals. It seems inconceivable that a clinical research project would not be connected with the clinics. To use the problem of attempted suicides as an example, they occur while the patient is on the ward, in the clinic and after the patient is discharged to the community. Any research team into such a problem should also include staff from the clinic.

5. There is one area in which there has been a remarkable degree of co-operation between the wards and the Aftercare Clinics. It is preceptoring.\* The meeting ground is the preceptors' conference. Although the emphasis is on training, a number of problems taken up demonstrate clearly the ward-aftercare continuum. When the unit preceptor ends his story with the patient's discharge from the ward, the supervisor from aftercare picks up and carries the story forward through the clinic stay.

Amid the satisfactions and frustrations of working in an aftercare setting, there is the thought of the future and an attempt to project possibilities of growth and development of the aftercare concept. When we follow the evolution of the mental hospital, isolated and behind thick walls where people were "put away," we notice that one aspect has been a steady progression of events

\* In The Mount Sinai Hospital, Department of Psychiatry supervisory psychiatrists are called Preceptors.

that lead to supplying the patient with a life that would resemble life in the community outside the hospital. Work, recreation, home visits, therapeutic community, are some aspects of this trend. An open door policy and psychiatric departments in general hospitals have made the boundaries between the outside and the inside even more fluid. The next step seems to be in the direction of supplying the necessary treatment in the community without taking the patient out of his milieu.

I recently read a report on psychiatric care in certain parts of Nigeria by Raymond Prince (5). There, among the Yoruba tribes, psychiatry never left the community level and the local healer, who specializes in the treatment of psychotics, does all his therapy either in his or his patient's hut. It seems that in our western civilization the care of the psychiatric patient had to go through the stages of first incarceration and then removal and isolation. But the trend is definitely toward community level care and not only by the psychiatrist but also by the non-psychiatric practitioner.

It is not accidental that we prefer to use the term aftercare services instead of Aftercare Clinic. At this point, we have many therapeutic services at our disposal at the clinic level, ranging from powerful drugs, psychotherapy in its many forms and variations, casework, ambulatory ECT, recreational therapy and occupational therapy. I would like to see all these services enlarged in scope and depth. But there is more that aftercare can do. It could become a pool of services for former psychiatric inpatients of this hospital and their families which would bridge the gap between life in the community and life in the hospital. What I envisage here is even a further blurring of the boundaries between in- and out-patient care or even between patient and non-patient. But the greatest gap aftercare could fill is the gap in the treatment cycle itself, between aftercare and forecare. It seems that the future of aftercare lies in the challenge of not only how to restore function on the family and community levels but how to help maintain function in the face of individual, familial and social stresses. This is an enormous task that would require tremendous resources. But couldn't we do something on a modest scale? I can think of a few measures that could be tried out on a tentative basis.

1. Home Visits. In selected cases, a team of doctor and caseworker would visit the patient's home, explore the home and family situation. This would be included in the program of treatment planning for the patient and family members.

2. Treatment of Relatives. When psychiatric treatment of a significant relative is indicated, this could be done in the Aftercare Clinic as part of total family treatment program. At this point, relatives in need of psychiatric treatment are referred to other out-patient department psychiatric clinics.

3. Unified Aftercare System. At this point we have four aftercare services in the Department: Child Psychiatry, Adult Psychiatry, Adolescent Psychiatry and the Day and Night Center. Child Psychiatry has to be kept apart

because of special and basic differences. The other three services may stand to benefit from unification. A study of such a possibility would be indicated.

4. Co-operation with Other Psychiatric Facilities in the Community. When more prolonged treatment is required, the wards and the clinics send their patients to city or state hospitals. Upon discharge from these hospitals most of them request aftercare in this hospital. Each patient is considered individually and if we have something tangible to offer he is admitted. We have developed important relationships with several state hospitals and Bellevue Hospital which are helpful when institutional care is needed. We even had a situation where an inpatient in a state hospital came to us for regularly scheduled psychotherapy. Sometimes several institutionalizations are necessary before a patient can find his level of functioning in the community. Co-operation with other community psychiatric facilities is of great importance to our own functioning.

#### SUMMARY

The aftercare psychiatric services of The Mount Sinai Hospital, Department of Psychiatry, are in a state of flux due to a marked increase in the number of patients. In an attempt to cope with a challenging situation, multiple avenues of approaches are required. Some have been suggested. What is also needed is a clearer philosophy of aftercare treatment as an integral part of total psychiatric care. Emphasis on patient function rather than personality change seems to offer good possibilities.

The greatest promise in aftercare lies in its ability to prevent recurrence of acute illness. This must be attempted on the family and community levels and in co-operation with other psychiatric facilities. A widened concept of aftercare is called for.

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# Hypogastric Artery Ligation and Its Value in the Control of Pelvic Hemorrhage

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Despite recent advances in the reduction of maternal mortality, hemorrhage remains one of the foremost causes of death in the childbearing period. In patients who have pelvic operations, serious blood loss is also a major cause of death. The ability to control bleeding in either the obstetric or gynecologic patient is often difficult or impossible with the known standard procedures. Even with the availability of blood, the recognition of the various hemorrhagic diatheses and the prompt treatment of hemorrhage by massive transfusion, fibrinogen, vasopressor or vasodilator agents may not be sufficient to save a life. Under those unusual circumstances, therefore, some additional methods are needed. It would seem that the revival and use of hypogastric artery ligation would offer a simple, safe and effective mode of dealing with this serious complication.

To emphasize the efficacy of the procedure, a review of the literature, a demonstration of the anatomical and surgical features, as well as the study of the experience of the gynecologic-obstetric service at the Sinai Hospital, was undertaken.

## REVIEW OF THE LITERATURE

Similar to the employment of early ambulation and the use of the transverse abdominal incision, ligation of the hypogastric arteries for the control of unabated bleeding is not new. All of these procedures were previously advocated, used, found to be satisfactory, but eventually discarded for reasons not clear. Over half a century ago, Kelly (1), Pryor (2) and Kronig (3) ligated the hypogastric arteries to control hemorrhage after spontaneous or operative bleeding in genital carcinoma. More recently, Decker (4), Reich and Nechtow (5), Siegel and Mengert (6) as well as others (7-12) advocated ligation of the internal iliac vessels for serious hemorrhage in the obstetric-gynecologic patient. Reports of those authors have done much to refocus attention to a valuable aid to those who perform pelvic and obstetric operations.

## ANATOMIC AND SURGICAL CONSIDERATION

### *Anatomy*

According to Cunningham's textbook of anatomy, the hypogastric artery in the fetus is a direct continuation of the common iliac trunk. This extension supplies numerous branches to the pelvis. It runs upward on the anterior abdominal wall and is prolonged to the umbilicus through the cord to the pla-

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centa. When placental circulation ceases, the umbilical portion atrophies. The permanent hypogastric (Fig. 1) is a short vessel, frequently longer on the right than on the left side. It arises from the common iliac, opposite the sacroiliac articulation, and then descends into the pelvis to terminate opposite the upper border of the sciatic notch into an anterior and posterior division. Each

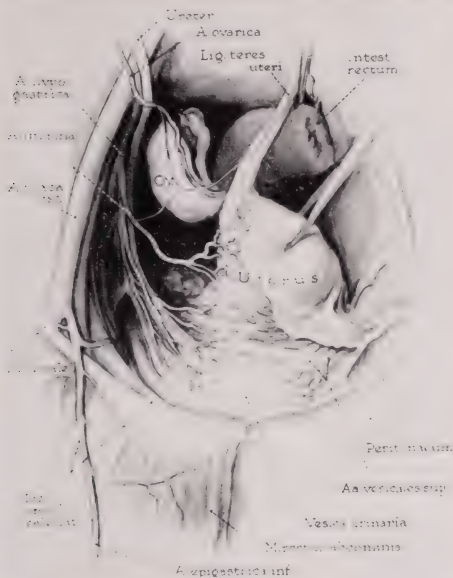


FIG. 1. The hypogastric artery and its branches.

From Anson, B. J.: *An Atlas of Human Anatomy*. Philadelphia, W. B. Saunders Company, 1950.

artery is covered anteriorly and medially by peritoneum. Behind the peritoneum lies the corresponding ureter which descends along the anterior border of the artery. The pelvic colon lies in front and to the medial side of the left vessel, while the terminal portion of the ileum bears the same relationship to the right artery. Posteriorly are located the veins, the lumbosacral plexus and the sacroiliac joint. The branches of the two divisions are as follows:

#### Posterior

##### 1. Ilio-lumbar

2. Lateral sacral
  3. Superior gluteal
- Anterior
1. Obturator
  2. Inferior gluteal
  3. Internal pudendal
  4. Inferior vesical
  5. Superior vesical
  6. Middle hemorrhoidal
  7. Uterine
  8. Vaginal

According to Shafiroff and his co-workers (12) the secondary blood supply to the pelvis consists of collateral pathways to both trunks of the hypogastric arteries, and an extensive circulation across the midline. When one of those arteries is occluded, blood supply is quickly restored across the midline from the opposite side. Ligation of both vessels eliminates this cross circulation, blood flow then depending upon anastomosis from the lumbar, the middle sacral, the superior hemorrhoidal and from branches of the external iliac artery. The supply from those channels is limited, and temporarily the effect is that of oligemia. However, in experimental animals, Diadrast injected into the abdominal aorta above bilaterally ligated hypogastric arteries revealed that there was adequate supply to the pelvic organs and tissues through well formed anastomoses.

### *Operations*

The transabdominal approach is preferable although the extraperitoneal operation is acceptable. This intraperitoneal exposure allows the hypogastric vessels to be ligated through a single incision, and inspection and treatment of pelvic pathology is possible. With the patient in Trendelenburg position, the abdominal cavity is entered through the midline. The bowel is packed off high in the peritoneal cavity so that adequate exposure of the posterior peritoneum is obtained. This structure is then incised for 7 to 8 cm over the common iliac vessel. The ureters usually remain attached to the medial peritoneal flap. The hypogastric arteries are thus visualized, identified and dissected free from 2 to 3 cm. An aneurysm needle with 2 silk or nylon sutures is passed around the vessel, one close to its origin and the other 2 cm distal. Both of the sutures are tied and the artery divided. Careful consideration should be given to the terminal portion of the ileum, the ureters, the superior hemorrhoidal artery and rectum, as well as the hypogastric veins and obturator nerve.

### SOURCE OF MATERIAL

The lifesaving potential of hypogastric artery ligation was amply demonstrated in a survey at the Sinai Hospital. From 1958 to 1963 inclusively, there were 24,754 obstetric and 12,583 gynecologic admittances. The material was



amassed from statistics supplied by the Admittance Office, questionnaires to staff members, and Record Room charts. In this manner nine examples of vessel ligation were found; three from the ward and six from the private service (Table I). Since the training of house staff is essential in this particular technique, it is gratifying to note that since 1958 the residents have had an opportunity to observe and perform this operation. This study showed that uncontrolled bleeding in both obstetric and gynecologic patients responded well to hypogastric artery ligation when the conventional methods of control failed. In the obstetric series two patients reacted promptly to the procedure. However, in one the bleeding continued and did not stop until the uterus was removed. The assessment of the gynecologic patients was also of interest. Prophylactic

TABLE I  
*Patients Who Had Hypogastric Artery Ligations*

Type of Patient	Diagnosis	Initial Operation	Number of Transfusions
Obstetric Patients	Ruptured uterus. Previous cesarean section	Total abdominal hysterectomy	14 units
	Abruptio placentae	Cesarean hysterectomy	20 units
	Postpartum hemorrhage	Curettage. Total abdominal hysterectomy	8 units
Gynecologic Patients	Endometrial carcinoma	Abdominal hysterectomy	13 units
	Dysfunctional uterine bleeding	Total abdominal hysterectomy	13 units
	Fibromyomata uteri	Total abdominal hysterectomy	3 units
	Carcinoma of cervix, stage I	Radical hysterectomy	4 units
	Prolapse of uterus	Vaginal hysterectomy	6 units
	Carcinoma of cervix, stage III	Radium and X-ray	18 units

ligation was done on two patients who had carcinomatous lesions, on the basis that the contemplated operation would be attended by severe blood loss. The remaining four patients were operated upon from ten to twelve hours after their original operation. In retrospect, the delay occasioned by vaginal packing and attempts to locate and suture bleeding points was not justified since continued hemorrhage might have exposed the patients to irreversible shock.

The transabdominal approach was used seven times. In the two instances where the extraperitoneal technic was used, difficulty was encountered in exposure and identification of the vessels.

Following is a short summary of the history of each patient on whom a hypogastric artery ligation was done.

### *Obstetric*

*Case 1.* This 25 year old woman entered the hospital with a rupture of the uterus in the 29th week of a twin pregnancy. Her previous delivery had been by

classical cesarean section. Upon admittance, a total abdominal hysterectomy was done immediately. Several hours later it became evident that there was intraperitoneal bleeding. Therefore, twelve hours after the original operation a second laparotomy was done. Profuse bleeding was found behind the bladder flap and as no specific point of hemorrhage could be located, a right hypogastric ligation was performed. She received 14 units of blood. The postoperative course was complicated by wound disruption on the eighth day. Her recovery was uneventful after a secondary closure.

*Case 2.* This 24 year old woman entered the hospital at the 38th week of pregnancy, with signs and symptoms of premature placental separation. Because of fetal death, an attempt was made at vaginal delivery. Oxytocic stimulation proved to be unsuccessful and since the symptoms progressed, a classical cesarean section and total abdominal hysterectomy were done. She continued to bleed despite the control of hypovolemia and afibrinogenemia. After 20 units of blood and 8 grams of fibrin, a bilateral hypogastric ligation was carried out twenty-six hours following the initial procedure. The bleeding ceased and the recovery was uneventful.

*Case 3.* A 30 year old patient entered the hospital with profuse bleeding ten days after a vaginal delivery. A curettage failed to control the bleeding. Because of the continued hemorrhage both hypogastric arteries were ligated. However, twelve hours later the profuse bleeding persisted. A total abdominal hysterectomy was done. Upon section of the removed uterus, fragments of retained placental tissue were found.

### *Gynecologic*

*Case 1.* This 43 year old patient had a total abdominal hysterectomy for dysfunctional bleeding. She re-entered the hospital in shock ten days after her discharge. Vaginal packing did not stop what was thought to be bleeding from the cuff. At exploratory laparotomy further attempts were made to control hemorrhage by suture of the right parametrial tissue and right ovarian vein. This proved to be unsuccessful, and a second operation was done at which time both hypogastric arteries were ligated. Her recovery was uneventful.

*Case 2.* A 44 year old patient had an elective total hysterectomy for fibromyomata of the uterus. After operation she became hypovolemic and failed to respond to drugs or transfusion. At operation, bleeding was found in the right parametrial area, and was readily controlled by right hypogastric artery ligation. Postoperative course was uncomplicated.

*Case 3.* This 45 year old woman had a radical hysterectomy for stage I carcinoma of the cervix. Both hypogastric vessels were ligated prophylactically at the time of operation. Postoperative course was benign and she left the hospital as improved.

*Case 4.* This patient was 63 years of age. She had a stage III carcinoma of the cervix. In the course of her treatment by radiation, vaginal bleeding became more profuse. The control of the hemorrhage was accomplished by hypogastric artery ligation through the extraperitoneal approach. She recovered

from the operation but died within a short time from generalized carcinomatosis.

*Case 5.* This 63 year old patient was operated on for fibromyomata uteri. Upon entering the abdomen, however, she was found to have an extensive adenocarcinoma of the endometrium. Prior to the hysterectomy both hypogastric vessels were ligated. Although the postoperative course was stormy, she recovered; but returned to the hospital six months later with widespread metastatic lesions.

*Case 6.* This was a 43 year old patient whose original operation was a vaginal hysterectomy and anterior and posterior colporrhaphy for uterine prolapse. The day she left the hospital, she began with profuse vaginal bleeding. An attempt was made to suture the cuff at exploratory laparotomy. The bleeding remained unchecked, and at a third operation a bilateral hypogastric artery ligation was done, extraperitoneal. Postoperative course was uneventful.

#### DISCUSSION

Postpartum and postoperative hemorrhage are serious complications that require immediate attention. The lack of control in unabated bleeding may result in rapid death. When the standard methods of treatment have failed, it becomes essential to resort to other means without delay. The ready availability of blood has produced a false sense of security. All hypotension is not necessarily associated with blood loss, and transfusion may cause dangerous hypervolemia (14). In addition, even the single blood transfusion is known to cause death in approximately one of every 150 patients, 40 years of age or over, as the result of serum hepatitis (15). It does not follow that blood should not be used. Emphasis is placed on the avoidance of transfusion during anesthesia, a rapid evaluation of the cause of the hypovolemia or hemorrhage, and prompt definitive treatment.

A knowledge of the anatomical and surgical aspects of the vessels that supply the pelvic organs is essential for every physician who practices obstetrics and gynecology. When faced with uncontrolled bleeding that follows vaginal or abdominal bleeding, or after a pelvic operation, the ability to locate quickly and ligate the hypogastric arteries may mean the difference between life and death. The works of Decker, Reich and Nechtow, Siegel and Mengert, as well as others have contributed to the re-establishment of this procedure as one of the most important obstetric-gynecologic technics.

The Sinai Hospital study was limited in number. Despite this, the residents in the past six years have had the opportunity to perform the operation. Hypogastric ligation in this series was done transperitoneally and extraperitoneally, prophylactically and of necessity. In each instance the immediate result was excellent.

#### SUMMARY

1. The important contributions to the literature that pertains to hypogastric artery ligation have been reviewed.

2. The anatomical and surgical aspects of hypogastric arteries have been presented.

3. The results in a survey of nine patients at Sinai Hospital from 1958 to 1963 who had internal iliac artery ligations in the course of their treatment have been recorded.

4. Prompt operation is emphasized for the control of bleeding when the standard methods of treatment have failed.

#### ADDENDUM

It would be natural to question, whether or not, a successful pregnancy occurred in any patient who had a previous ligation of the internal iliac arteries. Given, Gates, and Morgan (16), as well as Reich (17) and Nechtow and Shinagawa (18), report successful intrauterine pregnancies without complication at the time of delivery.

#### ACKNOWLEDGMENT

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# Rotation of the Ts $\hat{E}$ and Ps $\hat{E}$ Loops in Routine Vectorcardiography: Simple Method of Determination

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## INTRODUCTION

Attention recently has been focused on analysis of the Ts $\hat{E}$  loops in routine vectorcardiography in an attempt to enhance the diagnostic value of this procedure in clinical cardiology (1). However, as recorded customarily, the Ts $\hat{E}$  loop is of such small magnitude as to impede accurate study. Even after magnification of the Ts $\hat{E}$  loop, the direction of rotation in many instances cannot be visualized. Since direction of inscription is vital in the interpretation of vectorcardiographic loops, a simple method for determination of direction of rotation is proposed. This consists merely of increasing the duration of time between drop-shaped signals from 2.5 milliseconds to 10 milliseconds.

## METHOD

The method of recording vectorcardiograms in our laboratory consisted of a Sanborn amplifier model 185A with an attached Frank lead selector, and a companion Sanborn Viso-scope 169A. Photographs were made with high speed Polaroid film #3000 utilizing a Fairchild-Polaroid oscilloscope recording camera—model F296. For routine recordings, photographs were taken at  $\times 10$ ,  $\times 5$  and  $\times 2$  magnifications in the frontal, right sagittal and horizontal projections for both the cube and Frank systems. Loops were inscribed with "tear drop" shaped signals so that the larger end of the drop represented the front end, i.e., the blunt end of the drop faced toward the direction of inscription of the loop. For routine use, duration of time between drop-shaped signals customarily has been 2.5 milliseconds. All  $\times 10$  and  $\times 5$  magnifications were recorded at this time signal frequency. For better visualization of the direction of inscription of the Ts $\hat{E}$  and Ps $\hat{E}$  loops, recordings were taken at  $\times 2$  magnification both with 10 milliseconds duration between signals (one drop every 0.01 sec.) as well as the customary 2.5 millisecond duration between signals (four drops every 0.01 sec.).

## ILLUSTRATION

An example of the application of this method is illustrated by the following case. It has been stated that in left bundle branch block, if the rotation of the discordant Ts $\hat{E}$  loop is in the same direction as the QRS s $\hat{E}$  loop, the T wave

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changes are secondary; whereas, if the discordant TsÊ loop rotates in an opposite direction to the QRS sÊ loop, the T wave changes are primary (2).

M. L. is a 57 year old woman with coronary atherosclerosis manifested by angina pectoris and congestive heart failure. The routine twelve lead electrocardiogram revealed regular sinus rhythm, left axis deviation and left bundle

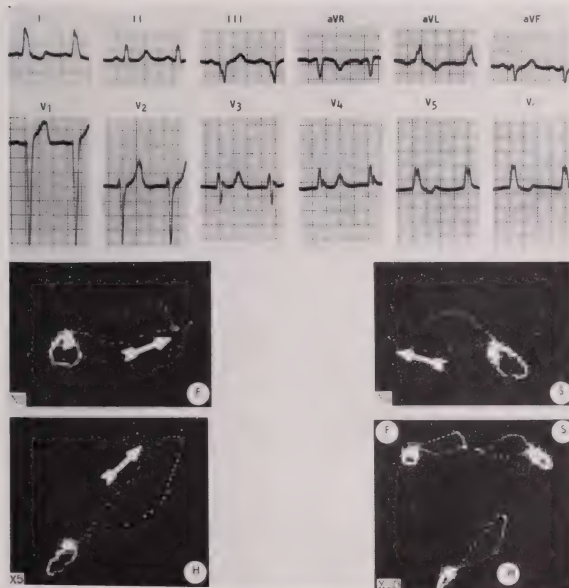


FIG. 1. M. L. f., 57 years. Arteriosclerotic heart disease with severe angina pectoris. Electrocardiogram and vectorcardiogram disclose patterns of left bundle branch block. Arrows indicate direction of QRS sÊ loops. Note that the direction of TsÊ loops cannot be discerned at amplification  $\times 5$  or  $\times 10$  with time signals inscribed at 2.5 millisecond intervals (= four timing signals per 0.01 second). F = frontal, S = sagittal and H = horizontal plane projections.

branch block. The QRS complexes were slurred, notched and widened to 0.12 sec.; the T waves were diphasic in leads I, V<sub>5</sub> and V<sub>6</sub> and inverted in AVL (Fig. 1). Routine vectorcardiograms taken by the cube system at  $\times 10$  and  $\times 5$  magnification with time signals recorded every 2.5 milliseconds showed the pattern of left bundle branch block. There was noted posterior orientation of the QRS sÊ loop associated with clockwise rotation in the horizontal plane, middle and late slowing of the QRS sÊ loop and a discordant TsÊ loop. Even

larger magnification ( $\times 2$ ) failed to reveal the direction of inscription of the T-S-E loop because the timing signals were partially superimposed (Fig. 2, Column 1). However, when the timing signal was slowed to once every 10 milli-

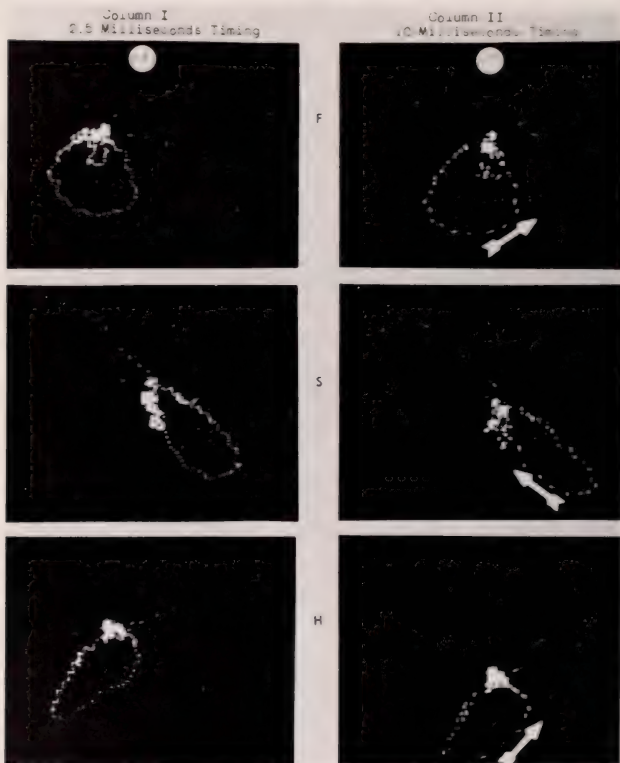


FIG. 2. Vectorcardiograms on same patient as figure 1; amplification is  $\times 2$  in both columns I and II. Column I—with 2.5 milliseconds timing: the direction of the QRS loop is seen clearly; the T loop direction is not discernible. Column II—with 10 milliseconds timing, direction of both the QRS and T loops is easily discerned (see arrows for T loop direction).

seconds (Fig. 2, Column 2), the direction of inscription became readily apparent (see arrows). In this case, the direction of the T loop rotation is opposite to the direction of QRS rotation in the horizontal plane and would, according to Nimura (2), suggest primary T wave changes.

## DISCUSSION

The importance of analysis of vectorecardiograph TS $\dot{E}$  loops has been emphasized recently (1). Relatively little had been written about the direction of rotation of the TS $\dot{E}$  loop because of the difficulty of visualization. One of us (P. S.) found that the direction of TS $\dot{E}$  loop rotation and frequently the direction of the PS $\dot{E}$  loop rotation could be determined readily even with amplification obtainable on the vector amplifiers in clinical use, by merely increasing the duration of time between the drop-shaped signals from 2.5 milliseconds to 10 milliseconds. That is, by recording only one drop shaped signal every 0.01 seconds instead of four signals every 0.01 seconds, one can separate the signals in the TS $\dot{E}$  and PS $\dot{E}$  loops well enough to visualize the shape of the signal and therefore easily determine the direction of inscription. Only one or two clear signals showing the front, blunt end is needed to show direction. This technique gives results which may be interpreted with ease, and no additional laboratory equipment is required.

Review of the literature reveals that Whipple (3), in 1952, when describing the interrupted drop-shaped time signal presently used in most vectorecardiographic recording devices, had suggested for the study of PS $\dot{E}$  and TS $\dot{E}$  loops a time signal frequently *slower* than the usual one signal every 2.5 milliseconds. Apparently, however, this technique has been neglected.

Other methods for determining the direction of PS $\dot{E}$  and TS $\dot{E}$  loop rotation have been utilized in the past. High speed motion pictures of the face of the oscilloscope (4), high speed magnetic tape recordings (5), and computers (6) can give this information, but these instruments are expensive and are not readily available. Automatic brightness circuits (7), selective unblanking instruments (8) and ordinary magnification of the loops are of some benefit, but these methods still may not enable one to separate the time signal well enough to determine the direction of rotation of the PS $\dot{E}$  and TS $\dot{E}$  loops. As magnification is increased above  $\times 2$ , the loops become unsteady on the face of the oscilloscope and therefore are difficult to photograph.

The technique of slowing the time signal frequency can be utilized on standard vectorecardiographic equipment commonly available in clinical laboratories. Although the direction of inscription of the TS $\dot{E}$  loop in general can be inferred from the fact that the initial portion of the TS $\dot{E}$  loop normally is inscribed at a slower rate than the terminal portion, the technique of slowing the time signal enables one to visualize the direction of inscription accurately.

We are at present utilizing this technique in all cases, in a comparative study of both normal and abnormal Frank and cube vectorecardiograms. This will enable us to determine the diagnostic value of the direction of inscription of the TS $\dot{E}$  loops in routine analysis of vectorecardiograms.

## SUMMARY

The importance of careful analysis of the TS $\dot{E}$  and PS $\dot{E}$  loops in clinical vectorecardiography is currently being emphasized. By increasing the duration of time between drop-shaped signals on a standard vector amplifier from the

usual 2.5 milliseconds to 10 milliseconds, one can separate the time signals in the TsÊ loop and frequently the PsÊ loop sufficiently to visualize clearly the shape of the time signal. At the usual 2.5 milliseconds timing between signals, it may be impossible to determine direction of these loops. Therefore, by slowing the time signal, one may readily determine the direction of rotation of the TsÊ and often the PsÊ loops.

Studies of the rotation of the PsÊ and TsÊ loops of vectorcardiograms taken with both the Frank and cube methods are being completed and the diagnostic importance of analysis of these loops in routine clinical vectorcardiography will be evaluated.

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## Alkeran as an Immunosuppressive Agent

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The number of cancer chemotherapeutic agents are increasing, but their efficacy leaves much to be desired. Recently Alkeran, a phenylalanine mustard derivative (P-(Bis-(2-chlorethyl)amino)phenyl)-L-alanine has been the subject of several encouraging papers on the treatment of multiple myeloma. A number of successfully treated cases have been reported (1, 2). The presence of large numbers of immature plasma cells in organs undergoing rejection has been well documented and their importance in the rejection process has been demonstrated by Kountz, *et al* (3). It was hoped that a drug capable of causing remission in a plasma cell proliferative disease might be capable of inducing enhancement of a homotransplanted organ.

The present study has been designed to evaluate the immunosuppressive capacity of Alkeran. The drug has been used alone, in combination with antimetabolites and together with antimetabolites and steroids at the time of rejection crisis.

### METHOD

The study includes twenty-eight female dogs weighing 17 to 25 kilograms. In a one stage procedure, a bilateral nephrectomy and splenectomy was performed, and a kidney from an unrelated donor was transplanted to the pelvis. The left donor kidney, irrigated with heparin solution (0.2 mg/ml), was placed in the right pelvis. The period of ischemia ranged from 15 to 40 minutes.

A) Nine dogs received Alkeran alone; they were pretreated for one to twenty days prior to surgery and received an average daily dose of 0.05 to 0.1 mg/kg.

B) Eight dogs received in addition to Alkeran either Azathioprine or 6-Mercaptopurine. They were pretreated for one day with both drugs. The average daily dose range of antimetabolite was 2.5 to 4.0 mg/kg. The average daily dose of Alkeran ranged from 0.08 to 0.1 mg/kg.

C) A third group of eleven dogs received Alkeran in doses ranging from 0.03 to 0.05 mg/kg. and either 6-Mercaptopurine or Azathioprine in doses ranging from 2.5 to 5.0 mg/kg. At the time of threatened rejection, cortisone or prednisone was begun. The criteria used to determine whether rejection was underway, were elevation of BUN, decreased urine output, or physical deterioration of the animal without apparent cause. Biweekly blood urea nitrogen, hematocrit, white blood cells, platelet counts and urine output were done on the

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The authors wish to acknowledge the assistance and advice of Dr. Fiorenzo Paronetto in the examination of the pathologic and immunologic material and in the preparation of this paper.

animals. At time of rejection, blood urea nitrogen was measured more frequently.

# RESULTS

A) In Group 1 (Table I) 4 of the 9 dogs died prior to transplantation. All four animals died of fulminating infections such as bilateral pneumonitis. At time of demise, the white blood cell count was severely depressed. The longest

TABLE I

Number	Dog No.	Days Pretreated	Average Dose Alkeran mg/kg	Toxicity	Cause of Death	Survival in Days
1	4920	18	0.075	none	uremia	8
2	4955	12	0.075	none	"	5
3	4919	17	0.075	marked	—	demise prior to surgery
4	4956	17	0.075	"	—	" " " "
5	4893	20	0.075	"	—	" " " "
6	4892	20	0.075	"	—	" " " "
7	4736	1	0.05	none	uremia	renal vein thrombosis
8	4727	1	0.05	"	"	2
9	4751	1	0.1	"	"	7

TABLE II

Number	Dog No.	Days Pretreated	Average Dose Alkeran	Average Dose 6-M.P.	Average Dose Imuran	Toxicity	Cause of Death	Survival in days
1	4839	1	0.075	2.5		marked	uremia	7
2	4838	1	0.1	2.5		none	twisted renal artery	3
3	4813	1	0.075	2.5		none	uremia	10
4	4822	1	0.05	2.5		none	uremia	18
5	5205	1	0.03		3.5	none	ureteral obstruction	4
6	5195	1	0.03	—	3.5	none	uremia	7
7	5271	1	0.03	—	4.0	none	bowel obstruction	2
8	5064	1	0.04	—	4.0	none	sacrificed	4

survivor of those animals having renal transplant was eleven days. Four dogs showing severe rejection on pathological examination at the time of death had markedly elevated blood urea nitrogen. The last dog in this group died one day after operation with thrombosis of the renal vein.

B) In the second group of animals receiving Alkeran plus Azathioprine (Imuran) or 6-Mercaptopurine the longest time of survival was 18 days (Table II). All eight dogs in this group died in uremia with marked rejection of the transplanted kidney.

C) In the group receiving Alkeran, Imuran or 6-Mercaptopurine and steroids at the time of rejection, ten of the eleven dogs died from rejection of the



homograft (Table III). The longest survivor in this group was twenty-one days. The eleventh animal in this group (5087) died on the fifty-first following transplantation with a normal blood urea nitrogen (Fig. 1). The cause of death in this animal was intestinal obstruction due to a volvulus of the small bowel and presumably unrelated to the kidney transplantation. A biopsy of the kidney 21 days following transplantation showed intimal proliferation and a marked round cell infiltrate (Fig. 2). A biopsy of the transplanted kidney two

TABLE III

Number	Dog No.	Days Pre- treated	Average Dose Alkeran mg/kg	Average Dose 6-M.P. mg/kg	Average Dose Im- uran	Average Dose Pred- nisone	Toxicity	Cause of Death	Sur- vival in days
1	5087	1	0.03	2.5	—	3	none	volvulus	51
2	5091	1	0.03	4.0	—	3	none	sacrificed marked rej.	7
3	5169	1	0.04	5.0	—	2	moderate	uremia	10
4	5075	5	0.03	2.5	—	4	none	uremia	16
5	5134	5	0.03	—	2.5	3	none	uremia	11
6	5351	7	0.03	—	2.5	2	none	volvulus BUN 24	11
7	5192	5	0.03	—	5.0	2	none	uremia	14
8	5612	3	0.05	—	5.0	2	none	uremia	12
9	5611		0.05		5.0	2	marked	toxicity	15
10	4452	4	0.04	—	4.0	2	none	sacrificed uremia	22
11	4407	9	0.04	—	3.0	2	none	sacrificed uremia	16

days prior to the animal's death, *i.e.*, on the forty-ninth day after transplantation, revealed a marked improvement in the transplanted kidney as evidenced by decrease in the cellular infiltrate and a decrease in intimal thickening (Fig. 3). This dog, in the fifty-one days following transplantation, had three rejection crises, all easily controlled with steroids and with increase of the antime-tabolite dosage.

## DISCUSSION

Alkeran, a drug responsible for a greater degree of remissions and symptomatic improvement in multiple myeloma than previously tried drugs, appeared

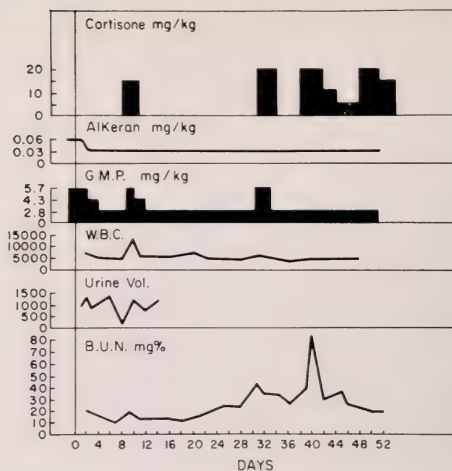


FIG. 1. Course and drug dosage—51 day survivor.

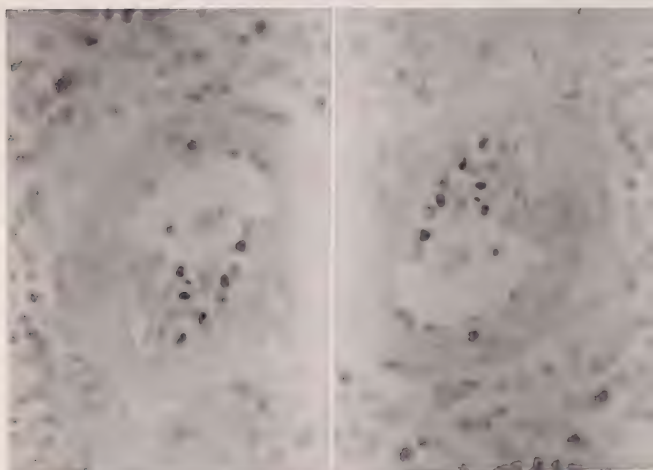


FIG. 2. Photomicrograph—renal homograft 23 days following transplant.

to offer no increase in survival of a transplanted kidney. The dosage used, much less proportionately than that administered to man, still resulted in toxicity. The establishment of a proper dosage in each animal was difficult and in no way correlated with length of graft survival. Dog 5087, which lived for fifty-one days following transplant, had three easily controlled rejection crises. However, investigators using Imuran and steroids without the addition of Alkeran have been able to reverse rejection crises. Zukowski was able to obtain a long term survivor in two animals, one receiving only steroids (5) the

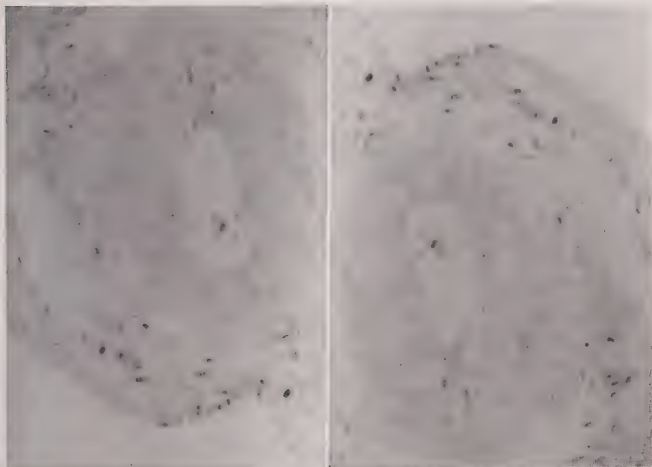


FIG. 3. Photomicrograph—renal homograft 49 days following transplant.

other only methylmercaptapurine (6). Pierce and Varco obtained a long term survivor using 6-Mercaptopurine (4) alone.

The studies referred to above describe at least one long-term survivor for 51 days. Such a single long-term survivor like others that have been reported probably represents a chance pairing of antigenically similar dogs rather than unusually effective immunosuppression.

#### SUMMARY

Renal transplantation was performed in twenty-eight dogs divided into three groups. Group I receiving Alkeran alone; Group II received Alkeran plus an antimetabolite; and Group III received Alkeran, antimetabolites and steroids at time of rejection crises. Of the 28 dogs, one died at the end of fifty-one days with a normal blood urea nitrogen, secondary to intestinal obstruction. The

remaining dogs died in uremia by the twenty-second day following transplantation. Therefore, Alkeran appears to have little or no value in preventing homograft rejection.

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## RADIOLOGICAL NOTES

CLAUDE BLOCH, M.D., AND HARVEY M. PECK, M.D.

### CASE NO. 251

This newborn male was the product of a normal pregnancy and delivery. At birth, the following abnormalities were noted on physical examination. The patient had a cataract in the right eye and an undescended right testis. Examination of the lower extremities revealed anterior dislocations of both knees. Both ankles were noted to have unusual flaxity and could be easily dislocated manually. There was also a cavo-varus deformity of both feet.

The family history was pertinent, in that the patient has a three year old sister who was born with bilaterally dislocated knees, bilateral congenital dislocation of the hip and a cleft palate.

Radiologic examination of the lower extremities with special attention to the knees revealed bilateral subluxations of the knee joints. The tibiae were noted to be anteriorly and laterally displaced in relation to the femora (Figs. 1A, 1B). There were no abnormalities of the hips.

After a preliminary trial of traction, the patient underwent an open reduction of the bilateral dislocation of the knees. The patient made an uneventful recovery.

### DISCUSSION

Bilateral congenital dislocation of the knees is a rare condition, usually noted immediately at birth, and with a strong family history of similar deformities. The exact genetic features have not been elucidated. Associated congenital anomalies are frequently noted. A case report is available where a woman with untreated congenital dislocation of the knees had three children by three different husbands. Two had congenital dislocation of the knee, and a third child had congenital dislocation of the knees and bilateral club foot and cleft palate and abnormal toes. Of 12 patients described by Neebauer and King (1), two had family histories with similar deformities. The condition is usually bilateral, but may affect only one knee. At operation, it is noted that in the majority of cases there is a subluxation rather than a dislocation of the knees with an anterior displacement of the tibia in relation to the femur with hyperextension of the knee. As opposed to congenital dislocation of the hip, where the primary abnormality is probably within the acetabular cavity, in dislocation of the knees the cartilages of the articulating surfaces of the knee appear intact. The tendinous insertions of the quadriceps are usually fibrotic with marked foreshortening. The treatment of choice is open reduction with plastic repair of the quadricep tendon. On occasion, simple traction is sufficient to correct the deformity.

*Case Report:* BILATERAL CONGENITAL DISLOCATION OF THE KNEES.

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Case 251, Fig. 1A. Examination of the lower extremities with special attention to the knees reveals bilateral subluxations of the knee joints. The tibiae are noted to be laterally displaced in relation to the femora. The hip joints appear to be normal with the capital femoral epiphyses in normal relation to the acetabulae.

Case 251, Fig. 1B. In the lateral projection, there is noted to be anterior displacement of the tibiae in relation to the distal femora.

Case 251, Fig. 1C. Examination in the frogleg projection again reveals the subluxation previously described with anterior slipping of the tibiae over the femora bilaterally. As noted, the leg can be extended cranially with the femora abducted. The hip joints are again noted to be normal.



## ACKNOWLEDGMENT

The editors wish to thank Dr. Robert Siffert for permission to publish this case.

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## CASE NO. 252

This 8 month old female was admitted because of a non-tender mass in the left parietal portion of the scalp. The patient, who had been the product of a normal pregnancy and delivery, had been entirely well except for the persistence of a swelling in the scalp since birth. At birth, two such masses were noted, one in the occipital portion in the midline, round and 3 cms in diameter, which was noted to be firm and immobile. This mass gradually disappeared within two months. The other was in the left parietal region and has not changed in size or shape since birth.

On examination, the only abnormality was limited to the scalp. There was a firm mass attached to the left posterior parietal region just above the parietal bossa. This measured 8 cms in diameter and was noted to be raised 4 cms above the surface of the skull. At its dome, there was a fluctuant area. The skin over the mass was normal. The mass in the left parietal bone was noted to be soft and fluctuant at birth but became quite firm at the age of two months.

X-rays of the skull revealed a smooth, rounded mass in the left posterior parietal region, measuring 8 x 5 cms in diameter. A thin rim of bone extending from the outer table of the parietal bone covered the entire mass in a shell-like fashion. This was noted in both the frontal and lateral projections (Figs. 1A and B). The remainder of the bones of the skull appear entirely normal. The sutures were normal.

At operation, the base of the mass was noted to be confluent with the outer table of the skull, so that there was no clear line of demarcation from normal outer table and mass. A thick membrane was noted to line the entire mass which contained murky yellow-green material and a few amorphous masses, undoubtedly representing old blood clots. The lining membrane stripped easily from the outer table and was removed. The bony covering of the mass was removed by rongeurs flush to the parietal bone. The patient made an uneventful recovery.

## DISCUSSION

Cephalhematoma in the newborn is common, with an incidence of approximately 1.5 per cent. It represents an encapsulated extravasation of blood between the peri-cranium and the external surface of one of the bones of the calvarium, frequently in the parietal region. The usual cause is trauma of the fetal head during labor. Fine linear fractures of the underlying bone can be



Case 252, Fig. 1A. Postero-anterior view of the skull reveals a 5 x 8 cm rounded mass over the left parietal bone. A thin shell-like calcification is noted covering the dome of the mass. A sharply delineated area of increased density is noted along the rim of the mass and at the junction of the shell with the parietal bone. This represents the cortex of the bone seen on end and does not truly represent a sclerotic area of bone.

found in about three percent of cases. X-ray findings in the first two weeks show a swelling of the soft tissues of the scalp without calcification. Near the end of the second or third week, bone begins to form under the elevated pericranium, first at the margin of the cephalhematoma but soon over the entire



Case 252, Fig. 1B. Similar findings are noted in the lateral projection. The lesion is noted to occupy the posterior portion of the parietal bone.

dome, forming a shell of bone. As in the case presented, the junction of the rim of bone with the underlying parietal bone causes a thin rim of increased density on the x-rays in both frontal and lateral projections. This merely represents the bony cortex seen on end and does not represent a sclerotic area of bone. Usually, the cephalhematomas are absorbed by the end of two weeks and rarely persist more than three months. When they do persist for years, the space between the outer table and the shell of bone becomes filled with diploic

bone. In the differential diagnosis, lymphangiomias and hemangiomias can present similar localized bony thickening. They are usually not present at birth and there is evidence of progressive growth with advancing age. Also, epidermoidomas and eosinophilic granulomas must be differentiated from cephal-hematomas.

*Case Report:* CALCIFIED CEPHALHEMATOMA IN A SEVEN MONTH OLD CHILD.

#### ACKNOWLEDGMENT

The editors wish to thank Dr. Sidney Gross for permission to publish this case.

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#### CASE NO. 253

This 11 month old child was admitted with an irreducible mass in the anterior abdomen. The patient had been known to have an asymptomatic umbilical hernia since birth. On the morning of admission, the mother noted that there was an irreducible mass within the ventral hernia. The patient was also noted to be febrile and irritable with normal bowel movements and no vomiting.

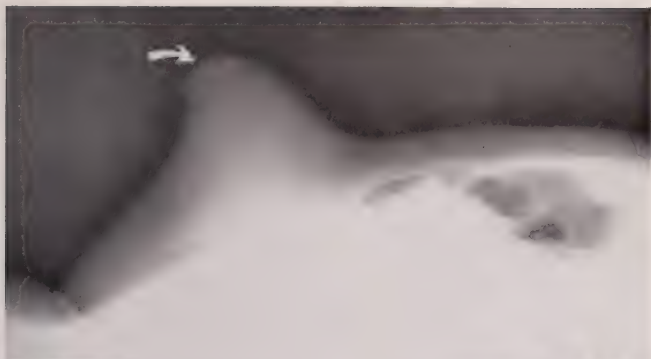
Positive findings on physical examination were limited to the abdomen where a 2 x 2 cm irreducible tender mass was noted under the umbilicus. Bowel sounds were active. Minimal tenderness was noted in the lower quadrants.

Laboratory examination revealed a mild leukocytosis with a normal differential. X-rays of the hernia in the lateral projection failed to reveal the presence of any gas-filled loops of bowel within the sac (Fig. 1A).

Because of the clinical diagnosis of an incarcerated umbilical hernia, the patient was explored on the night of admission at which time an umbilical herniorrhaphy was performed. No intestinal content was noted within the hernia. The patient tolerated the procedure well. The day after the operation, the patient was noted to have fever and an increase in leukocytosis with a white count of 45,000 cells and a shift to the left. During the next two days, the course was that of high fever up to 104° in spite of antibiotic therapy. Careful examination revealed right lower quadrant tenderness, and a flat plate of the abdomen taken at that time revealed a faint, rounded, calcified density above the right iliac crest which was interpreted to represent an appendicolith (Fig. 1B). Because of the x-ray and clinical findings, the patient was explored, and

Case 253, Fig. 1A. Lateral decubitus radiograph of the umbilical mass reveals no air-containing bowel within the umbilical hernia (arrow).

Case 253, Fig. 1B. Antero-posterior examination of the abdomen reveals no abnormal air-containing bowel loops. There are no soft tissue masses. Within the right lower quadrant, just above the iliac crest, there is a faint rounded calcific deposit (arrow) which represents a coprolith.



A



B

Fig. 1A-B.

an appendiceal abscess with gangrenous appendicitis was found. Appendectomy and incision and drainage were performed. The patient had an uneventful post-operative course.

#### DISCUSSION

This case is unusual in that the patient had two separate diseases; one, an incarceration of an umbilical hernia which is relatively infrequent and the second, an acute appendicitis with peri-appendiceal abscess. The latter diagnosis was not made at the time of admission and was only appreciated after the herniorrhaphy, when there was evidence of an infective focus within the abdomen in the post-operative period. The x-ray of the abdomen taken at that time, which showed a coprolith in the right lower quadrant substantiated the diagnosis of acute appendicitis with abscess. In a recent review of fecolith of the appendix, Dr. Fagenburg stresses the fact that when an appendiceal coprolith is found in a child with abdominal symptoms, the diagnosis of acute appendicitis with abscess formation is almost a certainty (1). Appendicoliths occur in approximately 12 to 15 per cent of cases with appendicitis with abscess and are of enormous help in the differential diagnosis of acute abdomen in children.

*Case Report:* APPENDICOLITH WITH ACUTE APPENDICITIS AND ABSCESS AND INCARCERATED UMBILICAL HERNIA.

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## Functional and Organic Mental Disorders in the Elderly

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"Functional and Organic Mental Disorders in the Elderly" is an area in the field of psychiatry in which I developed an interest many years ago, when it had less personal application than it now has. In the early 1930's, my interest was aroused inasmuch as I wanted to test out the then accepted point of view that the psychoanalytic approach was inapplicable to the treatment of psychoses and of the aged patient. Since I had been Clinical Director of McLean Hospital in Waverly, Massachusetts, I had the opportunity of working psychoanalytically with patients in both categories. It happened that two of my patients were both psychotic and in a category which I *then* considered aged, one being a woman of 56, the other a man of 60, both with depressions (1). Fortunately, both of these patients responded rather well to this therapeutic approach.

In 1939, I participated in a Symposium before the American Orthopsychiatric Association on "Old Age and Aging" in which I presented the psychoanalytic point of view, and it was emphasized at this conference that the aging person presented clinical pictures in all categories. "With advancing age certain pathological factors enter into the picture: namely, actual organic injury in the central nervous system, particularly the brain; and here we have clinical pictures which are characteristic and well demarcated as syndromes" (2). "Even though the organic defect as such is definitely based on destruction of the central nervous tissue and is characteristic to varying degrees of all diseases of the brain, whether arteriosclerosis or brain tumor, the content of the forgetting may have a definite individual psychological significance" (3). It was emphasized during these discussions that the emotional rigidity which one saw in elderly persons might very well serve as a defense mechanism and that it was reversible and, therefore, amenable to treatment. These observations are presented for the record to indicate that some of the problems that we now justifiably emphasize with this age group have been a matter of concern for sometime. This was recognized by Hippocrates several thousands of years ago in his "Airs, Waters, Places" (4) where he makes the statement: "old men have catarrhs because of their flabbiness and the wasting of their veins, so that some die suddenly, while others become paralyzed on the right side or the left."

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Read at The Jewish Home and Hospital for Aged. One Day Institute: Prevention of Disease and Disability in the Elderly, Thursday, September 24, 1964, Bronx, N. Y.

and in "Epidemics" (5) he says, "older people, and those whose natural heat is failing, have paralysis or raving or blindness."

With the actual and potential increases involved, including the recognition of the social, economic, medical and psychiatric features of these problems, a rather curious attitude seems to have developed among many workers in the field. There is a tremendous desire to do something and this has led to increased refinement of diagnostic methods, the re-evaluation of therapeutic techniques, and to a certain extent a minimization of therapeutic nihilism. On the other hand unfortunately, there is a kind of demand for action on behalf of the elderly which tends to minimize the fact that aging, either physiological or pathological, is a fact of life and that certain processes by and large do take place and lead to certain consequences. If this is so, the older person is not the younger person. Whether the sequence follows the seven ages of man in every individual or not, it is nevertheless true, that there is a sequence and the effects of aging must be taken into account if we are to look at the problem as physicians should from a sympathetic medical point of view.

An interesting research program was carried out at the National Institute of Mental Health. Its results were published in "Human Aging" (6), edited by James E. Birren. An attempt was made to obtain a group of individuals 65 years and older, who were to be optimally well, living outside of institutions, and who were to be studied theoretically from both physiological and psychological, as well as sociological and economical points of view. The goal was to find a group of some 50 such individuals who were free of illness of any kind. This proved to be a relatively impossible task and it eventually had to be divided into Group A, the optimal health group consisting of 27, and Group B of 20 with mild asymptomatic diseases. In the optimal health group there were such findings as healed chronic retinitis, osteoarthritis of the spine, right bundle-branch block, decreased hearing acuity, mild, diffuse EEG abnormality, diverticulosis of the esophagus, mild pulmonary emphysema, and so forth. In the second group, one found auricular flutter with enlarged heart, bronchiectasis, labile hypertension, angina pectoris (normal EKG), diabetes mellitus, mild, (controlled by diet alone), rheumatoid arthritis, to name a few.

Since certain studies have indicated that the psychological syndrome in this age group seems to relate to, and be weighted by other forms of somatic illness, this becomes of significance in the evaluation and understanding of the patients. In "Human Aging" in the chapter on "Psychiatric Aspects of Adaptation to the Aging Experience" (7) it is pointed out that 28% had psychoneuroses and 62% presented diagnosable functional psychopathology, including personality disorders. There were mild reactive depressions in 19% and in 23% there was diagnosable psychopathology including depression, even though the existent psychopathology was not associated with serious impairments since none of these subjects sought treatment, nor was treatment indicated. Nevertheless, psychiatric problems existed.

Karl Bowman (8) in his discussion on "Types and Special Factors of Mental Illness in Old Age," in 1937, indicated that "in proportion to the number of

people (60 and over) there is more mental disease. We, therefore, say that mental disease is in one sense a disease of old age. There is an increased fatigability, even though many can continue to adjust to life in a normal fashion, memory does become impaired. The individual is less stable emotionally." He also emphasized certain symptoms which, if they occur in this age group, most likely indicate organic brain disease. Arteriosclerosis is essentially a disorder of old age and, of course, so is the so-called senile psychosis. He ended his article on a rather pessimistic note by saying, "there is, of course, no specific treatment for the changes of old age. The machinery is worn out and we cannot supply new parts."

There are certain economic, sociological and psychological factors that begin to play a definite role in the individual who is in the process of growing old. In the female, the menopause period has a significant role to play in addition to the endocrinological aspect. Biologically it signifies the end of the childbearing period. Psychologically there is evidence that this is a time when there is a recrudescence of earlier conflict situations, with at times an attempt at resolution.

Retirement, which in our present culture begins to take place somewhere between the ages of 60 and 65, brings its own problems. There is a change in status at all levels and relatively few people can really gain the necessary satisfaction of their needs through hobbies and other forms of recreational activity. There frequently is a waning of physical capabilities at all levels. There occurs a series of objective losses with accompanying grief and bereavement. This is the time when the obituary column is turned to first in the daily newspaper, or in reverse is deliberately never read. Friends, relatives, colleagues begin to pass away and with each such passing one's own dissolution approaches. It may become increasingly difficult to form new attachments, new friends and to cathect new objects. These are but some of the psychological factors that begin to play a role.

I believe that the great change in attitude toward these patients has occurred and, indeed, the Jewish Home and Hospital for the Aged where this discussion is taking place, deserves a great deal of credit for this more optimistic and realistic point of view. I refer particularly to the work of Goldfarb, Sheps and their colleagues under the leadership of Doctor Frederic Zeman. The work of this group is outstanding in this area. It has already been stated that a wide spectrum of psychiatric syndromes may be present in the aging group. Many of these patients have a history of either continuous or episodic psychoneurotic difficulties. In the area of psychosis, late life depressions are common with or without a previous history. However, the vast majority of patients, 65 and over, who have been admitted to our Psychiatric Service at The Mount Sinai Hospital, suffer from either acute or chronic organic mental syndromes. These sometimes represent diagnostic problems of a complex nature and attention has been drawn by many authors to the need for a sophisticated diagnostic procedure. Sencer, for instance, in a recent paper on "Problems in Diagnosis of Intracranial Disease Among the Aged," concludes that "it is difficult to determine the etiology of

cerebral disease of the aged on the basis of mode of onset, clinical course, and basic laboratory procedures, including plain x-rays, electroencephalography and lumbar puncture. Complete neurological investigation, including pneumoencephalography and or angiography, is feasible in the aged. Potential morbidity must be weighed against the value of information to be obtained. Each case should be decided individually. Elderly patients who develop relatively rapid alterations in mental functioning are highly suspect of having gross structural changes due to neoplasm—either primary or secondary, subdural hematoma, infection, toxic-metabolic disturbances, as well as intrinsic vascular disease" (9).

Friedman and Bressler (10) in a number of papers, reporting their experiences in a general hospital's geriatric outpatient clinic, emphasized the presence of definite organic conditions of chronic type involving every system of the body. Of 139 patients, 12 suffered from chronic brain syndrome associated with cerebral arteriosclerosis with depressive features. It is of interest that the largest number of patients, 67, were diagnosed with psychoneurosis, depressive reaction with other types of depression playing a prominent role (11).

Suicide is a problem. Gardner (12) and his group have an interesting comment in this connection which says that the rate of attempted suicide is relatively low in the older age group as compared to the younger age group. But if an attempt has occurred, it portends successful suicide, usually within the first year after the attempt. This they take as an indication that the attempt occurs after an acute illness or a distress.

Each one of us usually presents a problem in terms of his own experience and, since ours is an experience connected primarily with the psychiatry department in a general hospital, the following presentations have been selected to illustrate the types of problems we are confronted with, the approach to them, and our therapeutic program.

A.B., a 63 year old widowed lady, was admitted to the hospital. She had been living alone following a stroke which left her with a left-sided hemiparesis, some six months later. Her husband had died of myocardial infarction some two years before. The patient had sold her house and moved into an apartment. Six months before admission, she had come upon her daughter in a rather compromising position with a boyfriend, and almost immediately developed the left-sided C.V.A. She was treated in a hospital and returned home after a month. She blamed her illness on her daughter, with whom she had not been getting along. The relationship was so poor that finally the daughter moved out. She began making demands on her two older married children. When these were not met, she wrote a suicide note, and took an overdose of sleeping medication, after calling her eldest daughter. An attempt was made to manage the patient at home, but she developed pneumonia and was admitted to a hospital where a tracheotomy was required. On recovery from the pneumonia, she was transferred to a state hospital. She was signed out, against advice by a psychiatrist, to return home with private duty nurses around the clock, while awaiting admission to The Mount Sinai Hospital. There was no previous history of psychiatric difficulty. Past history indicated she was born abroad, had relatively little educa-

tion, and had managed to work herself up to the management of a business of a professional nature. There she met her husband, whom she had hired as an employee. The relationship to her husband and three children always presented difficulties. She had very few friends and spent most of her time working. Some fourteen years previously, on account of menorrhagia, she had received radium treatment to her uterus.

On admission, she was neatly dressed. She cried a great deal, wringing her hands and pounding her fist. She had an obvious hemiparesis involving her left arm and left leg and was in a wheelchair from which she could get in and out of only with assistance. There was no obvious speech difficulty. The patient was greatly depressed, with weight loss, anorexia and difficulty in sleeping. She denied any problems and insisted in a loud voice that she was happy and had no "angry feelings" toward her family and that she had had a wonderful life. She denied any suicidal attempt, and refused to discuss the suicide note stating that she did not remember any such thing. She denied that she was suicidal or depressed in any way. Not only did she have no insight into her problem, but denied there was any problem.

Physical examination presented neurological signs of left-sided hemiparesis. Her blood pressure was 160/90. There was weakness and atrophy of the muscles of the left arm and the patient had a slight wrist drop and a moderate contracture of the left elbow. There was atrophy and weakness of the muscles of the left foot. The left plantar response was upgoing. There was no evidence of a facial paralysis and no clinical evidence of aphasia. The patient had a large decubitus ulcer about 3 inches in diameter and 1½ inches in depth with bone protruding at the medial portion of the right buttock. There were no other positive physical findings.

*Course in Hospital:* Her physical problems were managed in such a way that she received daily treatment for her decubitus ulcer plus daily physical and rehabilitation therapy. Her depression was treated with psychotherapy plus Elavil 80 mg/day plus Stelazine 8 mg/day. During the discussion with her therapist, she was able to face the realities of her difficult situation. Physically, she improved and was able to carry on her activities including her daily shower. She was finally discharged nearly four months after hospitalization on Elavil and Stelazine, and sent to a hotel until the family found an apartment for her. She was to continue her physiotherapy and arrangements were made for visiting nurse service. In addition, she was to be followed in our After-Care Clinic.

This patient presented a rather complicated problem and her improvement could be related to a number of factors, one of which was certainly removal from her apartment to a hospital, where a rounded out program relating to all aspects of her illness was carried out, whereas the psychotherapy remained at a superficial level, nevertheless, it enabled her to objectify some of her problems. The effects of Elavil and Stelazine on this patient undoubtedly played an important role in relation to the attitude of her children toward her which also seemed significant. Discharge diagnosis: 1. Psychiatric reaction. 2. Decubitus buttock. 3. Left hemiparesis.

Another case was that of C.D., male, age 70, divorced pharmacist. The patient



was admitted to the hospital upon the advice of a friend, because of increasing worry, depression, and feelings of boredom, loneliness and he found it more and more difficult to concentrate and to deal with people and began to spend more and more time by himself. He noted increasing difficulty in getting to sleep and would take long walks by himself. His appetite decreased and he became uneasy and irritable. He felt the future looked bleak, that his financial situation was perilous and although he had no suicidal ideas the future looked hopeless. The patient consulted a number of physicians who could find nothing wrong with him. After two days at another hospital, he came to The Mount Sinai Hospital. In this patient, precipitating factors seemed to be the death of his younger brother two years previously and invalidism of his older brother with heart disease. In 1963 his son had married against the father's wishes. The patient also had financial difficulties due to the falling off of his income.

His physical and laboratory findings were negative.

*Course in Hospital:* Initially the patient regressed considerably. He expressed paranoid ideas about his food and insisted that his transfer to a state hospital was imminent. He became confused staying by himself and required aid with his shaving, etc. He was seen almost daily in psychotherapy in addition to being placed on 400 mg Thorazine o.d., 100 mg Elavil, o.d., and Artane, 2 mg o.d. Under this regimen his depression lifted, paranoia disappeared and sensorium cleared remarkably. His judgment improved so he could make certain necessary decisions on his own. When his son came to visit him, the patient felt protected and "that someone cares." The patient was discharged apparently completely recovered and made arrangements for continuing psychiatric care. Until that time he is being followed in our After-Care Clinic and continued on drug therapy.

Final diagnosis: Psychotic depression reaction. During the course of his hospital stay, he presented evidence of an acute organic mental syndrome.

E.F., age 85, was a retired engineer whose chief complaint was an acute agitation with confusion, paranoid and suicidal ideation of two days' duration.

*History of Present Illness:* For the previous several months the patient was unable to concentrate on reading and experienced episodes of disorientation and loss of memory for recent events. He became angry easily and threatened to throw himself out of the window. Two days before admission, the patient became agitated and accused his wife of "plotting" against him in order to kill him. He had difficulty in recognizing his family members. His agitation increased at night but was somewhat alleviated by Stelazine, 30 mg in 24 hours. He still had episodic periods of confusion during which he made attempts to jump out of the window. There was no history of any previous psychiatric difficulties. His professional career had been rather successful. On admission, he was bedridden, emaciated, too weak to get up. He appeared friendly and co-operative, in spite of his suspicions. He spoke slowly but spontaneously. He was generally coherent and relevant, but with episodes of irrelevancy. His mood was somewhat depressed, but affect was appropriate. His memory for recent events was disturbed and became disoriented as to time and person. There was no derealization or depersonalization, no suicidal ideation. His physical and neurological examinations were essentially negative. There were a series of



psychotic episodes which cleared up rather quickly with Stelazine. He gained in strength but continued with many physical complaints. The patient was seen daily for frequent brief psychotherapeutic interviews and was placed on Stelazine and Elavil. There was some difficulty in arranging for his final discharge from the hospital with a question as to whether he should go to a nursing home. His improvement at all levels was excellent. Several days before discharge, there was a transient right facial paralysis which cleared up.

He was discharged from the hospital in good physical and mental condition to live at home. This patient's improvement seemed rather remarkable. No single factor could seem to account for it. The combination of excellent nursing care, continued relationship to the physician and the ward personnel in addition to the drug therapy might certainly be related to the improvement.

The three case histories are briefly presented to demonstrate reversibility and to emphasize that such reversibility and return to adequate functioning is not only possible, but happens. Undoubtedly other participants in this Institute will present many other of the complex aspects relating to the problem of the aged who are ill. In each instance, as has been emphasized in the literature and in clinical practice, many factors have to be taken into account in order to reverse symptoms and to maintain an adaptive equilibrium for the patient and his family which will result in a continuation of acceptable functioning.

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# The Use of Selected Phenothiazines in Elderly Patients: A Review

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There have been few specific studies on the applicability of phenothiazines to psychiatrically ill elderly patients. Lifshitz and Kline listed about 27 in the English language in a paper published in 1963 (1). There have also been few reviews of these studies or of that work relating in part to the older patient (2-5). We do know, however, that the geriatric patient responds in satisfactory ways to these major tranquilizers. This review will attempt to correlate several reports of the effectiveness of phenothiazines on the elderly patient. It will also point out the particular vulnerabilities and precautions necessary when working with the older patient. The paper will be divided into two parts: first; representative studies of often selected phenothiazines will be reviewed under dimethyl, piperidine, and piperazine headings, and second; a discussion will follow which will point out some general rules which can be used for these drugs when prescribing to the older patient.

## DIMETHYL PHENOTHIAZINES

### *Chlorpromazine*

Pollack found behavior improved in 79 per cent of 200 patients using up to 200-300 mg per day in patients who were noisy, aggressive, and restless. Anxiety and hostility were dominant. Fifty-five per cent of those with psychotic symptoms improved significantly (6). Wolff used doses of 150-200 mg per day in more than 1,000 agitated, excited, and hostile geriatric patients. He noted effective results in most within two to three weeks with maximum effect after three months. Side effects occurred in less than two per cent of the patients (7).

Howell *et al.* used chlorpromazine in 79 patients with senile psychosis, some depression, and anxiety. Forty-three (52 per cent) obtained significant benefit in doses up to 75 mg per day with side effects rare (8). Lifshitz and Kline reviewed thirteen studies which emphasized the use of chlorpromazine in the treatment of disturbed elderly and accumulated 677 patients. They noted that about 70 per cent had a favorable response rate. Most of these patients had brain syndromes with senile brain disease or cerebral arteriosclerosis (7).

It hence appears that chlorpromazine is a useful and reasonably safe drug when prescribed to elderly patients. Doses can go up into the 200-300 mg range without excessive risk. Wolff feels it is the phenothiazine of choice. A discussion of its side effects will be given in Part II.

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*Promazine*

Kent and Gitman used promazine (Sparine) in 452 patients over a three-and-a-half-year period and found satisfactory improvement in 88 per cent. Diagnoses were varied with disordered behavior as the apparent target symptom. The doses given were 25 mg one to three times per day with increments of 25 mg per day as indicated. The highest dosage used was 1.0 Gm per day for six months or more. Side effects occurred in four per cent of the patients. Fifteen experienced drowsiness, one became stuporous, two had a skin rash, and two Parkinsonism which did not abate when the medication was stopped. The commonest diagnosis was chronic brain syndrome, associated with cerebral arteriosclerosis. Concomitant psychotherapy seemed to increase the benefit obtained from the medication (9). In an earlier paper, they summarized their findings as showing that promazine is valuable and safe in controlling behavioral disorders of some aged persons (10).

Kozlowski used Miltown and Sparine in twenty-six chronic brain syndrome patients with psychosis and behavioral problems and found 81 per cent showing improvement. No side effects occurred except two episodes of fainting. Blood pressure decreased in all patients 10–20 mm of mercury. The dosage was 1.2 Gm of meprobamate and 150 mg of Sparine per day. Fourteen of sixteen patients who were hallucinating stopped by the sixth day. It was felt that the use of combined therapy tended to diminish the incidence of side effects (11).

Promazine is a less powerful, minimally hepatotoxic drug. Dose can range up to 1 Gm per day. 300–500 mg per day would be an average range for excessively anxious elderly patients.

## PIPERIDYL PHENOTHIAZINES

*Thioridazine*

Judah *et al.* in an early study tested 25 geriatric patients with Mellaril over a period of fifteen weeks. Eighty per cent of his patients were schizophrenic. He matched these against fifteen control cases. Doses ranged from 75 mg to 500 mg and later 700 mg per day. It was felt the medication increased socialization. There was one case of drowsiness and three mild cases with Parkinson-like features, one of which required Artane (trihexyphenidyl HCL). No other complications occurred (12). Kral used 20 to 200 mg per day in thirty-seven aged psychotics in need of tranquilization. Twenty-six were brain syndrome patients and eleven were functional psychoses. Seven had depressive symptoms and two manic ones primarily. Treatment averaged 217 days. No Parkinson syndrome, akathisia, or dyskinesia was produced. The patients were less sleepy than with chlorpromazine. Seventy-five per cent of the patients benefited so that they could either participate in group activities or could co-operate with nursing personnel. Fifty-eight per cent of the senile and sixty-three per cent of the nonorganic patients did well. Only 28 per cent (four of fourteen) of the psychotics with cerebral arteriosclerosis did well. The depressed ones were given

imipramine (Tofranil) in addition to thioridazine. It was emphasized that hypotension could be avoided or alleviated by using a low dose range and, therefore, only moderate dosages are advised (13). Jackson gave an average of 300 mg per day up to as much as 900 mg daily to 110 geriatric in-and-out-patients suffering from psychotic disorders primarily. One hundred and two were psychotic and eight neurotic. He felt that thioridazine alleviated the causal anxiety so that patients were either improved or markedly improved in ninety-eight cases. He found no serious case of hypotension. Mild extrapyramidal symptoms occurred in five patients and responded easily to benztrapine methanesulfonate (Cogentin). Four patients developed a rash. He noted no serious side effects (14).

This is a valuable, usually non-Parkinson producing, drug with satisfactory anti-anxiety and anti-psychotic properties. It is often the drug to switch to in the presence of continued anxiety and extrapyramidal phenothiazine complications.

#### PIPERAZINE PHENOTHIAZINES

##### *Acetophenazine*

Hamilton and Bennett studied this relatively weak piperazine in nineteen aged hyperactive psychotics. Eight patients received a placebo control. Nineteen of the total had organic brain disease with psychosis and seven were schizophrenics. One had a depressive reaction with psychosis. Dosage was from 40 to 60 mg a day. Sixty-eight per cent (thirteen of nineteen) of the patients who received Tindal showed a significant decrease in their hyperactive behavior. Nine of the fourteen with brain syndromes and psychosis improved. Three of four schizophrenic patients improved. Hyperactive behavior began to subside in two to ten days. Giddiness occurred in 37 per cent in the first week and subsided spontaneously in three to four days. Five of nineteen (26 per cent) developed excessive drowsiness (15). Sheppard *et al.* in a controlled study selected fifteen chronic paranoid schizophrenics, average age seventy-three years, and found a significant decrease in paranoid symptoms of delusions of control, of reference, and of persecution at a dosage level of 80 mg daily. Side effects of dizziness and weakness appeared with daily doses over 120 mg (16). Witton and Hermann treated 41 elderly male psychotics in doses of 30 to 60 mg per day with improvement in 78 per cent. Hypertensive responses occurred in five cases (17).

This is a valuable antipsychotic agent in doses of 40 to 60 mg per day. One can prescribe it if hepatic and hematopoietic complications are present. The blood pressure should be watched for hypertension.

#### TRIFLUOPERAZINE

Hamilton and Bennett administered Stelazine to eighteen apathetic elderly brain syndrome patients and found little improvement in management in doses of four to eight mg per day. Incontinence, confusion, and lethargy were unaffected. Side effects were frequent. Eleven of eighteen (61 per cent) developed marked lethargy. Forty-four per cent had drooling and difficulty swallowing and thirty-nine per cent had generalized muscular rigidity. In some lethargic

patients aspiration occurred. One patient had an increased number of seizures. No hematopoietic or hepatic effects were noted (18). Brooks and MacDonald tried Stelazine in twelve manic depressive or involuntal psychotic patients who were depressed. Their average age was 68 years. The usual daily dose was 10 mg. Eight patients required benztropine methanesulfonate (Cogentin) for Parkinson-like symptoms within one to two weeks. All had received it after six weeks. Even after rapid reduction to two mg per day rigidity was not adequately relieved. One patient developed leukopenia. Eight of eleven had an unexplained drop in serum cholesterol. Only four cases improved in these resistant depressions (19). Wolff used Stelazine in twenty-four withdrawn apathetic brain syndrome or schizophrenic patients aged sixty to eighty-one years in doses of 12 mg per day. Side effects occurred in two with drowsiness and Parkinsonism. Only six of the twenty-four improved (7). Lesse used Stelazine as part of a treatment program for agitated depressives in middle and old age. In doses of 9 mg, sixty-three per cent of his patient census of 100 improved. Side effects produced by the medications used were essentially limited to five patients with stiffness of the limbs which improved with Cogentin. Two patients had orthostatic hypotension which warranted stopping all drugs in the program (20). Post used up to 30 mg per day in seventy patients over sixty with paranoid persecutory ideation. A significant number improved after several months of treatment (21).

Stelazine is given to elderly agitated depressives in conjunction with a full treatment program. Doses up to about 10 mg per day are prescribed. Higher doses should be used with caution in view of the possibility of extrapyramidal complications. There is some evidence that it is effective over the long term, in paranoid conditions.

### *Prochlorperazine*

Shubin and Sherson used compazine in treating fifty aged patients with anxiety and some organic illness. The anxiety was considered reactive to the organic illness. Approximately 85 per cent showed a good response with relief of anxiety. The dosage was 20 mg per day. The only side effects were drowsiness in nine patients and an increase in agitation in one patient. The subjects became neater and cleaner. The highest dose was 40 mg per day (22). Settel gave 15 to 40 mg per day to 70 agitated geriatric patients with an overall effectiveness of 77 per cent. Side effects were mild and were relieved by lowering the dosage. There was no liver or blood toxicity. One extrapyramidal reaction ceased with a lowered dosage. When psychosis is present, as in twenty-three of his cases, 56.5 per cent can show improvement (23).

In doses up to 40 mg per day, prochlorperazine has a useful place in one's armamentarium for the treatment of agitation and psychotic phenomena in the aged. Risk of side effects in this dosage range is apparently minimal.

### *Perphenazine*

Busse has used Trilafon extensively in patients characterized by impulsive-ness, aggressiveness, assaultiveness, night wandering, and loss of sphincter con-



trol. They were schizophrenics, chronic brain syndromes, involutional psychotics with paranoid ideation, and paranoid states with intensified aggressiveness. He also uses it in tension and elated states. He has given up to 64 mg per day and has found it non-hypotensive with the Parkinsonian side effects easily controlled by lowering the dosage or by using Artane or Cogentin. He suggests not using any hypnotic agent with Trilafon since respiratory difficulties might occur (5). Ayd used 12 to 24 mg per day in twenty-five aged neurotics, paranoids, and others with satisfactory results. There is little lethargy and some extrapyramidal symptoms are easily relieved by Cogentin. He feels the incidence of this complication is high in ambulatory patients (24). Settel used 12 to 16 mg in ambulatory and 24 mg to 48 mg in hospitalized geriatric patients with anxiety and agitation. Good or excellent results occurred in 85 per cent with reversible Parkinsonism present in four out of five cases in which it occurred (25).

This is considered a valuable and relatively safe piperazine. Liver and blood complications are low but extrapyramidal symptoms can be expected to occur. Optimum dosage may be up to 24 mg to 32 mg per day.

#### DISCUSSION

There are several points which are important to consider when using phenothiazines in the elderly, in addition to the usual precautions and specific knowledge one has when using these drugs with younger patients. As one will see, they are not hard and fast rules, yet they can serve as guidelines for observation and future judgment when prescribing.

1. Hypotensive effects occur more often in the older more arteriosclerotic patient (26, 27). The incidence is higher with piperidyls and dimethyls than with piperazines (28). The probable autonomic effect of some phenothiazines may cause a lowered blood pressure. This is well known but should not serve as a deterrent to administration of needed medication. Some observation, perhaps by relatives, especially after initial prescription, is wise. In addition, the admonition to remain recumbent for an hour (29) or so after parenteral injection, an awareness of the blood pressure baseline, and an explanation of the possible hypotensive effect to the patient and relatives, if practical, may help in prevention and diminishment of anxiety. This also leads to the next step of suggesting to the patient that he can prevent dizziness, some weakness, and syncope by reclining when such symptoms seem about to appear. One should not overemphasize this. The hypotensive effect usually subsides after the first week (30) and should not interfere with further prescription. It is usually not important in bed patients. Lowering the dosage often lessens the hypotension.

2. Toxic-confusional reactions to the phenothiazines can occur with symptoms which make differentiation from cerebrovascular episodes and other toxic-confusional reactions a problem (31, 32). There is often just an increased brain syndrome of recent origin characterized by altered consciousness, poor perception of surroundings, excessive fear, and a fluctuating course, until the medication is stopped. It is best to stop giving that type of phenothiazine and switch to another category, i.e., from a dimethyl to a piperidyl. The presence of brain



damage from a previous stroke may enhance this possibility. In these cases, caution is necessary, with lower dosages needed, at least initially. Observation and a baseline neurological examination are important. These patients should have a temperature and pulse record kept should increased confusion occur.

3. There is some evidence that older patients may have a greater incidence of extrapyramidal signs (pseudo-Parkinsonism, akathisia, and dyskinesia) than younger people (33). They are more prone to the symptoms in the order given with dyskinesia least likely to occur. Most such phenomena occur in the first nine weeks of therapy (2). Dyskinesias usually present in the first five days. In some instances, Parkinsonism has persisted even after discontinuance of the medication, usually a piperazine (25, 9), although Hollister (30) disagrees that the phenothiazines are at fault. Differential diagnosis from "idiopathic" Parkinsonism or paralysis agitans becomes difficult. The cause-effect relationship in these cases is still in doubt. The treatment for such phenomena is usually discontinuance and switching to another phenothiazine in a different group, such as from a piperazine to a piperidyl thioridazine. Should this be impractical, lowering the dosage is valuable, if indeed this has not been tried before deciding to shift.

The use of barbiturates for pseudo-Parkinsonism is not wise in elderly patients. Often toxic-confusional states can arise secondary to the barbiturates; iatrogenic illness is compounded. In doses indicated: Akineton (biperiden) 1-2 mg twice a day, Cogentin (benztropine methanesulfonate) 1-2 mg twice a day, and Artane (trihexphenidyl hydrochloride) 1-2 mg twice a day, may be prescribed. Displacement of anxiety about prescription of phenothiazines to these agents should not interfere with their usage. Nevertheless, caution should be used in cases with glaucoma, prostatic hypertrophy, and cardiac and renal disease. They should be prescribed at meal times (34).

The attempted production of an extrapyramidal syndrome as an indication of, or correlate of, the therapeutic effectiveness of the medication is an attractive philosophy (35, 36), but is not yet proven (37, 38). There are added risks in using it in the elderly patient; nevertheless, one might consider it as possibly having value psychodynamically in cases of schizophrenia of late life (Roth) (39).

4. Obstructive jaundice is reported as occurring in 0.5 to 5.0 per cent of cases on chlorpromazine (6). Indications for routine alkaline phosphatase and total serum bilirubin levels (Van den Bergh), as well as other hepatotoxicity studies vary from author to author (40, 41) in studies based on younger patients. Some say it's probably best to have a total serum bilirubin done serially in the first sixteen weeks of therapy for medicolegal purposes. The jaundice oftentimes occurs in the first six weeks of therapy and clears up in two to four weeks after cessation of the phenothiazine medication. Chlorpromazine and the dimethyls are more prone to cause it, the piperazines less so (30). For interpretation of laboratory studies, a satisfactory medical history and physical examination are necessary. This is especially so since elderly patients, including those with brain syndromes and psychiatric complications, often have concomitant

physical illness of a significant degree (42). Indeed, Agate feels they go hand in hand (43). It seems probable that those patients with liver disorders have an increased chance of developing jaundice (30).

5. Blood dyscrasias can occur at any time although most authors state that in younger patients it occurs in the first four weeks. Whether blood studies should be regularly done is a moot point. Some feel they are not necessary, although others say they are valuable in the first eight or sixteen weeks (44, 45). There are reports that the prevalence of this side effect is greater in older female patients with or without brain syndromes. Indeed, Pisciotta reports that with chlorpromazine, middle-aged women are most susceptible to agranulocytosis in the first twelve weeks of therapy with an onset that is abrupt and with high fatalities. Two of the cases were women in their sixties. Needless to say, if a blood dyscrasia occurs, one should stop the medication, and often all phenothiazines, and initiate proper precautionary therapy with consultation as indicated. Thrombocytopenia and hypoplastic anemia are rare possibilities. These types of complications are less likely to occur with piperazines.

6. The initial drowsiness seen when prescribing dimethyl phenothiazines and thioridazine is not mentioned as occurring more frequently in older patients by Pollock and Kral. The latter speaks of the absence of usual drowsiness, although this is often present initially when prescribing thioridazine to younger adults. Ayd speaks of acetophenazine as having sedative properties. A good rule of thumb to follow is to recognize that with chlorpromazine, the dimethyls, and thioridazine drowsiness is in proportion to dosage. Also the initial week or so of prescription may result in drowsiness which usually subsides after this period. Sometimes unexpected deaths occur with excessive sedation, and asphyxia has been considered a factor (47, 48). It is often best to alter the diet and provide some supervision at mealtimes in these cases. Increasing the amount of coffee available may be helpful.

Chlorpromazine has the potential of lowering the convulsive threshold in epileptic and brain-damaged patients. Focal slowing may occur on the electroencephalogram (49). In these cases neurological consultation is indicated and appropriate anticonvulsant medication started. This is not a common occurrence. Skin complications, usually a sensitivity to sunlight, may occur in those individuals given the dimethyls and thioridazine. It is reported by Goldman as more common in the elderly individual (24). It usually occurs in the outdoors type and may not be bothersome to them. Lowering the dosage often relieves the symptom. Lifshitz and Kline feel that with chlorpromazine, this is proportional to dosage (1). The rash may respond to an antihistamine.

Autonomic phenomena such as nasal stuffiness and dryness of the mouth occur in the initial induction period. Reassurance that this will pass may be indicated. Palliative measures such as sucking on a lozenge may be suggested depending on the nature of one's relationship to the patient.

7. Many older patients may be on a Rauwolfia alkaloid for hypertension. It is often best to review the indications for reserpine with the prescribing practitioner. Phenothiazines are used with caution in such cases (50). Should depression occur, the phenothiazine is lowered or stopped. The phenothiazines are

not prescribed for a depressed mood and occasionally depression may arise from its use (7). Evaluation of its significance in comparison to the therapeutic effect of the phenothiazine is indicated, and a decision for continuance made on that basis. Often it is helpful to stop the medication to see if the depression is caused by it. Should depression be present *sui generis*, antidepressant medication may be given concomitantly with phenothiazines in selected cases.

8. Dosage is often a bugaboo in the elderly. The rule is usually to lower the regular dosage by half and increase slowly. The fear is only of such side effects as have been discussed above, i.e., hypotension, toxic-confusional reaction, excessive sedation, and perhaps extrapyramidal phenomena. Other complications such as jaundice and blood dyscrasia are considered to be independent of dosage. Paradoxical state, an intensifying of the symptoms, is sometimes considered psychogenic and is essentially also independent of dose. Hence, caution is indicated to be sure, but there is much lost in prescribing homeopathic doses. The object is to get a therapeutic effect on the target symptoms. It behooves the physician to try to obtain such a response up to the vagaries of the particular side effect that occurs (36) although the benefit derived from the occurrence of the side effect itself is open to question.

It is best to prescribe a lower than usual initial dosage especially in outpatients, and, in several days, increase at a usual rate until the limits of the dosage used in the reported studies. This is summarized under each of the selected phenothiazines in Part One. When prescribing above these dosage levels, and according to the above mentioned empirical and inferential philosophy, it is usually best to press on cautiously and at a slower rate. If the indications are present and no toxic reactions have occurred, one may give relatively high dosages: 75 mg of chlorpromazine was given TID I.M. to a psychotic agitated 76 year old woman who had paranoid delusional ideation, suicidal thoughts, and who threatened to leave the ward. With no response the dose was increased in 50 mg increments every two days until 100 mg QID was prescribed. At this dosage level, there was diminution in psychotic ideation, behavior, and hostility. Strict nursing observation could be dispensed with. This type of approach can perhaps be followed most readily in treating hospitalized patients.

9. In spite of the presence of much psychopathology, the target symptom effect of the phenothiazines should not be lost sight of. These medications are prescribed for their effect on anxiety, especially motor and autonomic components. It seems apparent that anxiety becomes an ideational concept if these aspects are not present. Hypochondriasis, and worry are diminished only to the extent that the presence of clusters of thoughts of self-concern and guilt are reacted to by anxiety. The ideation attached to anxiety about guilt is not affected directly.

The phenothiazines are also used for an anti-psychotic effect. This can occur in lower dosages and over shorter periods of time in elderly patients with brain syndromes. This is naturally quite valuable and helpful. The disordered ideation in acute brain syndrome, however, often remits in a relatively short period of time as the cause of the illness is treated.

Psychotic ideation in chronic brain syndromes secondary to arteriosclerosis

or senile psychosis may improve with phenothiazines. Observation and questioning about the psychotic ideation is indicated. Oftentimes there is a lessened verbalization of psychotic material but probing may show its presence. Confabulation secondary to impaired intellectual functioning should not be considered the same as delusional ideation and is not affected except in how a component of anxiety may be associated with it.

Its effect on rage itself is open to speculation; however, clinical experience indicates that a hostile mood can be altered. There is oftentimes diminution of control of mood in some brain damage patients; the motor elements of the fight aspect (51), according to Cannon's theory, are altered. The motivation to express rage is lessened, either biologically or through psychological factors. The element of aggressiveness, which is motivated by anxiety, is altered, usually in a favorable way.

Their influence on nocturnal variations of behavior is significant and usage has shown that this is helped quite favorably by phenothiazines. Chlorpromazine is perhaps the equal of chloral hydrate in helping bring on sleep. The latter medication may be given with phenothiazines, if indicated, in the usual dose (0.5-1.0 Gm.H.S.) (52). If the insomnia is excessive, then there is the possibility a depression is present which might require a different treatment program.

10. The value of these medications on intellectual functioning is minimal. They are not helpful in alleviating chronic intellectual impairment. The anxiety attendant to an acute brain syndrome may impair responses to the usual questioning indicating orientation, memory, judgment, comprehension, and fund of information. To the extent that this is present there may be an effect on the type of response to the medication. It is probable, however, that this would ordinarily resolve in the usual course for survivors in acute states.

Their effect on confusion, in that this is defined as an alteration of consciousness, usually fluctuating, with attendant phenomena indicative of lowered perception, is such that the behavioral and verbal aspects of such states of lowered consciousness is affected, often for the better, but the impairment itself is not. Chronic states of apathy, withdrawal, and loss of interest are not usually affected in elderly brain damaged individuals. The so-called stimulating effect of trifluoperazine is usually not a value in motivating such patients. Of course, a comatose or somnolent state is a contraindication to the use of phenothiazines for psychiatric purposes.

11. The manner of administration is important in these elderly people. Since isolation is often an important defense or unfortunate occurrence, the interpersonal relatedness of these individuals oftentimes depends on the few contacts they have. Their relationships with nurses and the psychiatrist are of overriding importance in evaluating, and perhaps influencing the effectiveness of the medication. The sensitivity of these individuals is well known (53), and oftentimes psychosis fluctuates with interpersonal situations (54). This is shown to be especially so in acute brain syndrome patients where anxiety is considered related to distorted interpretation of sensory images. Proper medical care plus alteration of environment with proper lighting, diminishing the amount of auditory

and tactile stimuli the patient has to perceive, and the presence of family with constancy of relationship and interpersonal contact is important and useful (55). Similarly, the manner of administration plays a part in such cases and in others in which interpersonal relationships are important. Several authors deem this quite significant, reasoning that the anxiety produced by an inability to cope with the environment causes symptoms such as attempts to manage the doctor and fellow-patients or seek aid from personnel in their immediate surroundings (56, 57). Indeed, it could be said that the responsiveness to these medications could indicate the reversibility of most symptoms according to interpersonal theory. This should be differentiated from placebo effect where oftentimes the sole usefulness of a medication is considered as resulting from essentially interpersonal attitudes (58). These medications are therapeutic in their own right.

#### SUMMARY

It seems possible to state that the use of some phenothiazines in geriatric patients involves the acceptance of precautions and the knowledge of accumulated data. This includes recognition that hypotension occurs more often than in the younger person; that agranulocytosis can occur; that the presence of a large amount of physical illness can complicate the diagnosis of side effects such as obstructive jaundice, and toxic-confusional symptoms may occur more often. In addition, the dosage range necessary for effecting target symptoms is often less than that used for younger adults. There is evidence that a Parkinson-like syndrome occurs more often than akathisia or a dyskinesia, and also more often than in younger people. Barbiturates for such complications are not indicated. Adequate baseline physical examination and follow-up is important in understanding and treating any side effects which might occur. In general, one might say that serious sequelae are rare, or at worst uncommon, and that in knowledgeable and thoughtful hands, the phenothiazines are effective agents for the treatment of psychiatically ill elderly patients.

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# **The Continuum of Adrenocortical Disease: A Thesis and Its Lesson to Medicine**

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For a number of years it has been recognized that adrenocortical insufficiency may be of varying degree and that there are probably all gradations from normal adrenocortical function to absolute insufficiency (1). This has been demonstrated by the responsiveness of the adrenal cortex to stimulation with corticotropin (ACTH). This variation in function has also been shown to exist in other organs which can be subjected to physiologic testing or loading (1, 2). More recently in a study of a form of the adrenogenital syndrome due to  $11\beta$ -hydroxylase deficiency first manifest in the adult woman (3) the significance of varying degrees of congenital enzymic failure present in the adrenal has become apparent to us. It has long been recognized that in congenital adrenal hyperplasia different degrees of enzymic block or deficiency may be present with regard to either 21 or  $11\beta$ -hydroxylation or even  $3\beta$ - or dehydrogenation but emphasis has not been placed on the continuum of the degree of deficiency which becomes apparent if the various reports in the literature are reviewed. In somewhat analogous fashion, it has been recognized that in non-adrenocortical disorders there may exist a spectrum of a disorder. Thus this continuum or spectrum of a disease affords a cross section of a disorder from which the time element has been eliminated. This latter dimension is introduced by following the course of any given patient with a progressive disease over a period of time. Such progressive impairment is physiologically demonstrable not only in the adrenal that is being destroyed by untreated tuberculosis but also, among others, in the kidney of the patient with increasing renal insufficiency, in the liver of subjects with advancing cirrhosis, and in the heart subjected to relentless destruction of the myocardium by inflammation or necrosis and fibrosis.

Thus in any given adrenal disorder one must consider the basic defect and its continuum and secondly the continuum effect that ensues with time. A congenital defect in adrenocortical  $11\beta$ - or even 21-hydroxylation may not become apparent till post-puberal life. Whether the defect increases merely with the passage of time or because the physiologic load is increased with aging is not as yet clear but both possibilities must be considered. In adrenocortical  $11\beta$ -hydroxylation deficiency it seems likely that the increased androgen production by the adrenal cortex due to the basic  $11\beta$ -hydroxylation defect augments secondarily the  $11\beta$ -hydroxylation deficiency (3).

The clinical picture of disease, then, is a reflection of a sort of vector effect of the continuum of disease from progressive functional impairment superim-

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posed on the spectrum of the basic defect. As emphasized in a prior publication (1) it is only with our advancing understanding of the underlying physiologic processes that this phenomenon can be comprehended.

We now must consider whether these observations are pertinent in adrenocortical hyperfunction. Since the congenital adrenogenital syndrome is due to the previously mentioned defects, it need not be considered further.

The variations seen in the adult form of the adrenogenital syndrome are also compatible with the concept of a continuum. Although the evidence at present is not definitive, it may be that all or many instances of the so-called adult form of the adrenogenital syndrome are really congenital in origin. In support of this suggestion are the previously mentioned reports of congenital adrenocortical hyperfunction first becoming apparent post-puberally. On the other hand it is also possible that in the absence of enzymic defects there is a congenital facilitation of the pathway in the adrenal cortex to androgen and estrogen (4, 5) which is first clinically apparent in the adult.

The problem of Cushing's syndrome due to non-tumorous adrenocortical hyperfunction is more difficult of resolution. It is apparent that we can induce Cushing's syndrome iatrogenically by the administration of sufficient quantities of cortisol or its analogues for an adequate length of time. However, it is of interest that in our present state of knowledge it is extremely difficult if not impossible to pinpoint the precise onset of Cushing's syndrome under such circumstances, due to the absence of exact criteria. The utilization of one or a group of the signs and symptoms seen in the classical spontaneous form of the disease is obviously unsuitable for this purpose. Spontaneous Cushing's syndrome must be viewed in this frame of reference. It seems unlikely that the disorder is an all-or-none disease. It, too, is probably a continuum from normality to the disease which we now recognize in the florid clinical state. In a study of 50 patients with Cushing's syndrome (6), we demonstrated that the incidence of the various symptoms increases with the duration of the disease and that certain features are observable only late in the course of the disorder. Using the principle of regression, it is apparent that the disease was probably present before we were clinically able to recognize it. Further if the degree of disease varies from individual to individual it is easily understandable how difficult it would be to recognize the mildest forms. If we then accept these concepts how can we expect to detect the mildest forms of the disorder by data (i.e., the dexamethasone suppression and the ACTH response test) obtained only from florid instances of the disease? It is already apparent that not all instances of florid Cushing's syndrome hyper-respond to the administration of ACTH and it has also become evident, as one might have predicted, a priori, that the suppression test is not infallible. Accordingly, the tests may fail us particularly in those instances when we are uncertain as to the presence or absence of the disease. At present the most acceptable physiological test for Cushing's syndrome is probably the cortisol secretion rate. It remains to be resolved whether there is a continuum of the secretion rate from the normal to the patient with florid Cushing's syndrome and if so what is the dividing line from normal. The basic

clinical or experimental reflection of this secretion is still unknown, be it an effect on the connective tissue or, even more fundamentally, on certain enzyme systems. At present, the best we can do is to demonstrate a relatively gross effect such as the former. Further, if we accept that one of the chief features of Cushing's syndrome is the effect on connective tissue and bone, is the phenomenon a reflection of hypersecretion of cortisol per se or does it actually reflect the cortisol-anabolic hormone ratio? In this regard it must be remembered that androgens are more potent anabolic agents than estrogens, a possible explanation for the preponderance of the syndrome is women. Nonetheless, a concept of a cortisol-anabolic hormone ratio makes the problem even more complex since even other hormones may be exerting effects on the connective tissue.

Our knowledge of primary aldosteronism is based on the clinical picture associated with tumor. Only more recently has the question been raised in regard to the incidence of non-tumorous primary aldosteronism particularly in relation to hypertension. On the basis of this concept of adrenocortical disease the prediction that a continuum of this form of adrenocortical hyperfunction will also ultimately be delineated does not seem difficult to accept.

Evidence can readily be adduced for similar concepts in regard to gonadal dysgenesis (Turner's syndrome), Klinefelter's syndrome, congenital thyroidal enzymic defects, and the testicular manifestation of the adrenogenital syndrome (7).

Although these concepts if true would be of great interest to the student of adrenocortical diseases as well as of endocrinology their greater importance lies in their application to disease in general.

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# The Concepts of Agnosia, Apraxia and Aphasia After a History of a Hundred Years

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The attempt to explain psychic phenomena in terms of the physiological activity of the brain concentrated, during the last century, largely on the endeavour to correlate psychological entities with the activity of isolated cerebral structures, i.e. to localize psychic syndromes to circumscribed cerebral (preferably cortical) areas. In these studies, the trinity of agnosia, apraxia and aphasia occupies a crucial position between "physiological" disorders (such as, hemiplegia or sensory loss) which appear localizable and disturbances of psychic phenomena (such as, mood, thought, memory, etc.), which offer great difficulties in distinct cerebral localization.

While there have been and still are great controversies concerning the nature, pathogenesis and even the existence of the syndromes of agnosia, apraxia and aphasia, there has been scarcely any discussion of the close correlation or even identity of their respective patterns. Some authors made painstaking efforts to explain one of these disorders in terms of the other, e.g., to distinguish agnostic and apraxic traits in aphasia, or to create such sophisticated syndromes as, for instance, "amnesia for color names," which allow unending and fruitless discussion about their belonging to agnosia or aphasia, or both. Despite great dissent as to the nature of these disorders, there seems to be unanimity regarding their structural similarity or, rather, isomorphy. This seemingly self-evident isomorphy, however, is inconsistent with some trivial but essential facts.

The concept of agnosia derives from Munk's experimental work with dogs. A dog, after a brain lesion, apparently had a visual disorder which Munk—correctly or not—interpreted as "mind blindness" or visual agnosia. Thus, it is seen that the first established case of agnosia was Munk's dog and not a human being.

Aphasia, on the other hand, has different problems associated with it, e.g., an American is not able to ascertain whether a German is aphasic or not unless he speaks his language. In other words, agnosia, whatever it may be, is a morbid state affecting an inborn faculty which man has in common with all higher animals. Aphasia, on the contrary, concerns an artificial system of communication which is not only unique to man but is also specific for single human societies and must be learned deliberately by each member of that society. So the fields in which aphasia and agnosia occur are absolutely different, and this difference alone should prevent the assumption of too close a relation between both states.

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The notion of agnosia depends upon the assumption of elementary sensory processes leading to elementary sensations which in second or gnostic processes are combined into structured perceptions. Sensory defects then would concern the first and agnostic disturbances the second of these two processes. Contrary of these hypothetical assumptions, psychological observation demonstrates that nothing like elementary sensations exist in our sensory experience. Rather, there are complex and structured perceptions which differ only in the degree of their complexity and differentiation. What were supposed to be elementary sensations are either insufficiently differentiated perceptions or noetic (intellectualized) abstractions of complex and structured perceptions.

In the tactile sphere, for instance, a simple touch is not an elementary sensation but a percept meaning: "A solid object at the tip of my finger which I cannot distinguish exactly." The "elementary sensation" of temperature means: "a warm object which I cannot further differentiate." In the visual sphere, a light spot means: "A bright object which I cannot clearly distinguish." On the other hand, a color is by no means an elementary sensation but an abstraction of the "category color" from a colored object, e.g., "red" from a "red" target in perimetry. The sensation of temperature may also be such a noetic abstraction derived from the perception of "a test tube filled with warm (or cold) water."

Since the identification of a separate gnostic act presupposes the existence of elementary sensations, discernible in structured perceptions, there is no justification for maintaining the notion of a gnostic act and, consequently, the notion of agnosia. Should there exist, contrary to the evidence on hand, such a special gnostic act it would be implicated in every sensory activity and make every sensory disturbance agnostic, whether it was the more complex patterns or the "simple" sensory defects seen in lesions of peripheral nerves or optic pathways.

Moreover, thorough analysis of sensory function and performance, using adequate methods, regularly demonstrates the so-called elementary sensory processes to be much more complicated than was supposed by the classical sensory physiology of the past century. Fascinated by the notion of elementary sensations, the physiological methods of examination of the sense organs were designed to give exact thresholds for such elementary sensations. To overcome the obvious difficulty in obtaining exact and constant thresholds, rigid standard conditions for examination were introduced. This trick tends to conceal the fact that these thresholds are not constants like those of the physicist, but rather, achievements produced and maintained by the activity of the nervous system. These thresholds are results of dynamic processes in which the time factor plays an essential role and must be adequately considered. Methods for examining the various sensory modalities that meet these new demands have been developed by us and by various other groups, especially Teuber and his associates.

If sensory activity is examined with adequate methods rather than by determining artificially chosen thresholds, a great variety of sensory dysfunc-



tions including a vast number of complex patterns of perceptual disorders is found rather than the simple loss of one or another fictitious "elementary sensation."

In the tactile field, it is then easy to demonstrate that the rare cases of so-called tactile agnosia are based on a functional loss of sensibility, generally of cortical origin, in the same way as any other tactile disorder.

In the case of visual agnosia there is more difficulty for several reasons. For example, contrary to the different tactile areas of the skin, the visual organ acts as a whole, generally under the leadership of its most efficient parts. The subdivisions of the visual organ (central and peripheral) participate in different visual performances to a variable degree and importance. Therefore, before excluding a simple visual impairment as the source of a seemingly agnostic disorder, we need to sufficiently test the whole visual sensory field. This is a hard task, especially if we take into account the temporal factor in each of these different functions.

We have tried to evaluate the role of the several aspects of visual function and the special patterns of the sensory deficit in the many different types of visual agnosia: object agnosia, simultanagnosia, prosopagnosia, spatial agnosias, etc. Reviewing the literature on this topic, one is surprised by the striking discrepancy between the rare and badly examined case material and the copious and bold theoretical implications attached to such poor empirical data.

The sensory loss which we find in such cases, however, does not account for the full range of symptoms ascribed to agnosia. In most cases in addition to the visual defect there is implied a general mental factor of one or another type; e.g., intellectual deficiency. In some instances the seemingly agnostic disorder may even be due exclusively to such general mental factors without a specific sensory loss. For instance, the traditional tests for so-called simultanagnosia are the well-known pictures of the Binet-Simon battery which is an intelligence test. And, for the same type of visual agnosia, Lange, in the *Manual of Neurology*, quotes as most instructive, the case of a patient of Head's but unfortunately that patient had a semantic aphasia. Among the patients with difficulties in spatial orientation (so-called visuo-spatial agnosia) there are some with true visual defects, e.g., functional restriction of the peripheral visual fields, while in other cases the spatial difficulties are part of a general defect, e.g. disorientation in the course of a mental disturbance.

These facts give the impression that agnosia, in the original sense of a disordered gnostic act, does not exist. Even in a simple pragmatic sense the concept agnosia is of no use because it does not imply a uniform pattern of dysfunction. Rather agnosia appears to us to consist of an arbitrary mixture of most diverse sensory or even non-sensory disorders grouped together because of superficial similarities. It could, at best, denote a certain type of clinical syndrome without implying cause, without explanation and of little if any use in analysis of the subsumed sensory deficits.

Passing over to the topic of aphasia, we find completely different facts and problems. One of the arguments against the existence of agnosia as a real

pathogenetic entity is the rare occurrence of pertinent cases. Aphasia, on the contrary, is a rather common state whose existence nobody would doubt. The difficulty here stems from major and evident differences between individual cases of aphasia.

This multiplicity of aphasic syndromes seems to argue against a simple and uniform aphasic disorder. The classical doctrine of aphasia therefore tried to overcome the difficulty by dividing these syndromes into different subgroups (e.g., motor, sensory, etc.) which were derived from the underlying theory of language functions. This theory was constructed on purely hypothetical grounds in analogy to agnosia and apraxia, with arbitrarily chosen partial functions of language corresponding to the respective morbid states. This theory was abandoned long ago because of its inconsistency with the empirical observations. Yet the system of classification has been kept, tacitly and unnoticed, in spite of Jackson's sarcastic criticism 90 years ago.

The essentials of this system of classification are still included in the seemingly non-committal distinction made by Weisenburg and McBride between expressive and receptive aphasia. Even this vague concept still carries two essential prejudices of the classical doctrine, namely, the strictly symmetrical derivation of speech from motor and sensory elements (analogous to apraxia and agnosia) and the tacit assumption that all the symptoms in these patients belong to the realm of aphasia.

Both premises—although far from being established—are of great significance. They fortify the tendency to overestimate the differences among the various types of aphasia (each type being conceived of as a discriminative essential) and, consequently, to neglect the features common to each of the various types of aphasia. Furthermore by explaining every detail of the clinical syndrome by peculiarities of the aphasic pattern, these premises block the search for non-aphasic factors which may also be involved.

Speech, when considered apart from any preconceived theory, is a most complex performance, which depends upon many different factors. Disturbance of any of these factors may disrupt speech activity. Thus we must carefully eliminate all factors except linguistic if we want to evaluate aphasia as a purely speech disorder. Among the nonlinguistic factors are, for instance, general intellectual capacity, mental activity and productivity, hearing, and, last though not least, the highly differentiated motor activity of articulation which is indispensable for speech.

To start with the motor impairment of articulation, we could demonstrate in so-called motor aphasia—in full accordance with P. Marie and many others—a genuine motor impairment of the articulatory muscles which we termed cortical dysarthria. Cortical dysarthria may occur combined with, yet is independent of, a true aphasic disorder. Since it is a special feature of spastic paralysis involving the major hemisphere, it is regularly combined with right-sided hemiplegia, right facial weakness and transient disorders of deglutition. Other than the defect in articulation, there is apraxia of the articulatory muscles (whatever the term apraxia may mean).

Cortical dysarthria, even when combined with aphasia, must be clearly distinguished from aphasia because both states are pathogenetically entirely different and vary independently of one another.

Dysarthria accounts for several symptoms which were ascribed to particular types of aphasia. Because dysarthric patients experience difficulties in the articulation of words they reduce their utterances to a minimum and, consequently, speak in telegram style. This telegram style is not a special agrammatic disorder and hence is not a linguistic defect but rather, a means to minimize articulation and is, linguistically, a surplus performance: for it is evidently more difficult to condense an information into a telegram instead of conveying it by a long letter.

Changes of the rhythmical and musical qualities of speech, described by Monrad-Krohn under the term dysprosody, also belong to dysarthria, whereas in aphasia, the prosodic qualities of speech are well preserved and in some cases even afford the patient a last means of communication as for instance, in severe jargon aphasia.

Cortical dysarthria is the most frequent and most important non-verbal factor in disordered speech, but there are still others to be considered, as for example, in some cases of aphasia a hearing impairment must be taken into account, particularly in those patients with marked difficulties in repetition. Such an impairment is also suggested by the close anatomical neighborhood of cortical auditory and speech areas. Unfortunately, we have so far been unable to establish this assumption because there exists no reliable non-verbal method for the checking of cortical auditory dysfunction.

Another evidence is easier to prove: "Amnesic" and "sensory" aphasia are generally thought to be two completely different types of aphasia with different—and in the case of amnesic aphasia somewhat nebulous—pathogenesis in the light of the classical doctrine. If we go into particulars the difference between both becomes less evident. The original idea of amnesic aphasia as an isolated defect in naming with normal receptive functions was conceived under a bias and does not withstand objective checking. Thorough examination reveals expressive and receptive defects in amnesic aphasia as well as in every other type of true aphasia. Therefore, the essential difference between both states is restricted to the logorrhoeic flow of speech with abundant paraphasias and unawareness of the numerous errors in "sensory" aphasia, as contrasted with the patient's taciturnity and the sensitiveness to his errors in "amnesic" aphasia. The patients with amnesic aphasia are overscrupulous and lacking in self-confidence (as a normal reaction to their alarming linguistic defects) whereas the different behavior of "sensory" aphasics is due to lack of self-criticism and to an euphoric mood, a common feature of organic brain lesion, independent of aphasia. Accordingly, the lack of self-criticism is not restricted to speech performances but bears upon the general attitude of such patients. Therefore, the difference between "amnesic" and "sensory" aphasia is not a difference in the linguistic defect, but is due to additional effects of the brain damage on general behavior. This fact also accounts for the temporal

evolution of symptoms which in progressive morbid states develop from an amnesic into a sensory type, whereas the recovery from sensory aphasia usually occurs over an amnesic state.

In cases where the focal lesion responsible for aphasia is combined with diffuse cerebral damage—for instance, cerebral arteriosclerosis—aphasia may be complicated by a reduction of general mental activity. This leads to “*echolalia*” where the patient tends to answer a question by changing the order of the very words of the question into a more or less correct reply. Other important factors modifying aphasic symptoms are a reduction of general intelligence, and the respective language as a linguistic entity which has essential bearings upon certain, seemingly aphasic symptoms. So, for instance, the much greater role which so-called “*agrammatism*” plays in German aphasias as compared with English-speaking ones evidently depends on the different grammatical complexity of both languages.

Even individual pre-morbid speech habits may be of influence on aphasic symptoms, e.g., pre-morbid literacy or the habit of fast-speaking, as we could demonstrate in some cases. In other cases which may be rare but do occur, there are emotional and personality factors to be taken into account. Aphasia means a severe psychological stress with most serious personal and social consequences. It is more than likely that such a grave emotional impact should—in one case or another—evoke personality reaction.

An example may illuminate this point:

A dental surgeon suffered a stroke at the age of sixty. According to the records of the general hospital where he received first treatment the initial symptoms consisted in a complete right sensorimotor hemiplegia and a severe “sensory and motor” aphasia. He made a quick but somewhat unusual recovery in so far as hemiplegia and sensory aphasia, as gauged by his receptive disorders, entirely disappeared within six weeks, whereas the motor aphasia, i.e., the inability to speak, did not improve at all, even during a special treatment in a speech rehabilitation center. Four years after the stroke, when we saw the patient, he uttered only unintelligible sounds while his understanding of language and his intellectual capacities were evidently undisturbed. He would have been the most perfect and most pure case of motor aphasia ever described—if only these symptoms had not been neurotic ones. His emotional repulsion against speaking was so strong that, in testing for oral apraxia, he perfectly executed silent movements (protruding of tongue, blowing up of cheeks, licking of lips, etc.) while he refused movements combined with the production of noise as whistling, coughing, smacking.

The key to this puzzling behavior was found in the patient’s biography which revealed a background of constant emotional stress. His wife was drug-addicted and lived in a mental hospital for 15 years. Despite a good income as dental surgeon the patient was in constant financial difficulties because he had to pay for his wife’s hospitalization—as long as he earned money. Very shortly after the onset of his illness and in striking discrepancy with his, so far, good recov-

ery the patient sold his office and his equipment and retired into a home for the aged people, leaving the care of his wife to public welfare.

Evidently, this patient had an aphasia at the beginning of his disease; yet, as the organic sequelae of the insult disappeared his inability to speak and, consequently, his professional disablement were maintained on a neurotic basis.

Such extreme cases are certainly not frequent although, personally, I know some more of them. But more frequently than generally considered emotional factors account for individual peculiarities in an otherwise organic syndrome.

Of such non-verbal factors influencing speech activity there exist, without doubt, still more which have not been considered at all. When we distinguish these additional non-verbal factors from the genuinely linguistic disorder (aphasia) and try to evaluate their effect on speech performances, it becomes evident that just these non-verbal factors account for the differences in the clinical pattern of aphasia which, in the usual classification, were attributed to different types of aphasia. Real aphasia, on the contrary, then appears as a uniform pathogenetic entity with a single dimension of language deficit (Schuell and Jenkins).

This aphasia proper approximately corresponds to the so-called "amnesic" aphasia. A sensible interpretation of this morbid state, however, has been spoiled by the bias of preconceived theories and by undue emphasis on the accidental traits in the morbid pattern prompted by the scheme of classification. Being of non-verbal order, however, these accidental traits must rather be eliminated for proper analysis of aphasia per se. This is especially true for the time-honored bias which assumes the opposition of expressive and receptive disorders, while real aphasia affect both aspects of language indiscriminately.

As soon as we abandon the sterile expressive-receptive dichotomy, it becomes evident that the pattern of aphasic achievements and failures depends on other determining principles, namely: on the linguistic significance of the respective performances. It has long been known, at least since Jackson, that only part of speech activities is affected by aphasia, whereas in another respect the disorder exceeds the field of articulated speech.

Regarding speech activities little affected in aphasia, it is also known since Jackson that otherwise severely impaired aphasics sometimes surprise by well-expressed greetings, conventional phrases, or curses in appropriate situations. Now, a curse is a display of emotion; and salutation, a stereotyped reaction to a certain situation of frequent occurrence. Both reactions are common components of animal behavior and do not really require human language. They could be easily replaced by clenching of the fist, or the shaking of hands.

To this category of speech performances also belongs, to a certain respect, logorrhoea, a distinctive feature of "jargon aphasia" with an abundant flow of meaningless phrases. This defect in speech is more related to the babbling of a parrot than to sensible human language. However, there is a surplus of speech



rather than a deficit. It is important for the interpretation of aphasia that the logorrhoeic chatter conveys no information about the concepts and ideas of the speaker, yet provides clear evidence as to his being pleased or angry, i.e., evidence of his emotional state. Such aphasic speech productions are similar to curses, which, also, have little linguistic content (usually a sacrilege or an offense against good manners) but much emotional substance.

In contrast to this, what is most impaired in aphasia is the production of linguistically coded information; or, to put it more simply, aphasia primarily affects the ability to inform about facts, i.e., to make meaningful statements. In the difficulty with the production of meaningful information, the aphasic disorder transgresses the domain of articulated speech. Deaf-mutes communicate by their "language of gestures." In aphasia they lose the faculty to express their ideas with gestures. However, the loss of "language of gestures" in aphasia is not restricted to deaf-mutes. Gestures used by aphasic patients when trying to make up for their linguistic shortcomings are quite vague and helpless, particularly if compared with the significant gestures of a non-aphasic deaf-mute. This observation caused Finkelnburg as early as 1870 to consider aphasia to be a kind of asymboly. The impairment in aphasia of other "significant" non-verbal performances, i.e., drawing and modeling, shall be considered later.

The comparison of preserved versus impaired modes of communication in aphasias reveals a most important fact: emotional reactions and stereotyped situational reactions are available to aphasias whether they are expressed in articulated speech or by non-verbal means. Conversely, the aphasic patient is unable to make a meaningful verbal or non-verbal statement. This confirms the statement by Hughlings Jackson, made nearly a hundred years ago, that the aphasic is "not unable to speak" but rather "unable to make propositions." In other words, the linguistic disturbance which we term aphasia is only the most conspicuous but not the essential feature of the syndrome. Rather, the aphasic disorder does not primarily concern articulated speech but the faculty to "make propositions," i.e., to express one's ideas by meaningful statements.

To make a proposition or a statement means to relate different concepts to each other in a certain logical connection. In the linguistic sphere statements are embodied in sentences, and concepts in such words that have meanings of their own, namely, nouns, adjectives, and verbs. This correlation permits a further differentiation and specification of the aphasic defect. It is well known that the aphasic difficulty mainly concerns those words with a definite conceptual meaning. These are either entirely lacking in aphasic language or distorted by paraphasias, contrary to the so-called "small words" which simply express logical relations and do not have a meaning of their own. The latter are undisturbed or constitute as in logorrhoea, a verbal surfeit surplus. As the words of the language cannot be separated from their underlying meaning, we may as well define aphasia as a disorder in the management of concepts and in the use of concepts for meaningful statements.

The concepts are the elements of conceptual thinking which endow the



human mind with its power of omnipotent and omnipresent imaginations and ideas and, by this means, allow it to transgress the boundaries of the actual situation (to which an animal is always confined). The concepts are categorical condensations and abstractions of many and various experiences and seem to be immediately and consistently present in the mind whenever needed. This quick and reliable availability leads to the consideration of concepts as being obvious and needing no further explanation. However, such an idea would be erroneous. When we consider a simple everyday concept as for example, that of a "tree," this includes the innumerable real trees of our experience together with all those which our imagination could add. We are provided with such a multiplicity of the most diverse representatives of the class "tree" that their common essence, the "concept of a tree," is restricted to a vague, colour-, contour-, and timeless phantom. In this vague and shapeless matter there remains only one substantial and stable thing which we can manipulate, namely, the word "tree" of the language.

This word "tree," however, stands for the whole range of potential meanings of the concept. In the individual case only a part of this full content is implied. So, the concept of a "tree" may mean an oak or a beech; it may represent a conspicuous object of the landscape, or one of the many elements of a forest, an economic product of forestry, or a botanical class, etc. Its actual meaning always depends on the context in which the word (or concept) is placed. The actual meaning of concepts used in language must be developed in a highly complex process of actualization occurring immediately and incessantly while speaking—whenever the words are to convey a sensible meaning.

Now, because aphasia primarily affects the management of concepts and their use in language, it seems worthwhile to consider the actualization of concepts in aphasic patients. For this aim, however, we must avoid one mistake which has repeatedly led past observers astray. Normally, one derives information concerning another's concepts through the channel of language. In the aphasic, this channel is disturbed and cannot, therefore, provide reliable information. The conceptual disorder of aphasia, however, is not confined to articulated speech and, therefore, non-verbal performances might implicate the actualization and application of concepts.

One such non-verbal access to the patient's concepts and their actualization is possible through drawing and modeling. If one draws (or models in clay) an object "from memory" (i.e., without model), he must take its shape from his "imagination," i.e., from his concept of the object. Regarding communication, such designs and models are "statements" about the shape of these objects. Drawing and modeling are performances which aphasics can perform. Yet, their products present remarkable deficiencies which sometimes supply valuable evidence about the nature of the underlying concepts. The following pictures are samples of such modeling by aphasic patients with plastic material.

Figure 1 is supposed to be a "canary." While it is definitely a bird, it is certainly not a canary; it resembles a chicken more closely. This is analogous to what one would call paraphasia in a speech performance: The correct word (or

concept in this case) is replaced by another one which belongs, however, to the same conceptual sphere (in this case the superimposed category of bird).

The next figure (Fig. 2)—from the same patient—is intended to be a "flower-vase." It surely belongs to the bowl—or dishlike vessels; yet it did not fully develop into a tall and narrow-mouthed flower-vase.

Another type of insufficient differentiation offers the "giraffe" (Fig. 3) from another patient. The specific discriminative feature of the giraffe may be termed

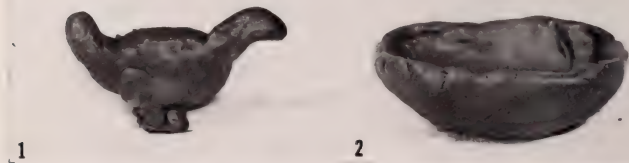


FIG. 1. "Canary."  
FIG. 2. "Flower-vase."

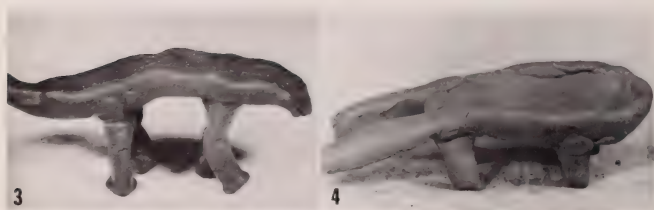


FIG. 3. "Giraffe."  
FIG. 4. "Wheel-barrow."

"oversize," namely, of its neck and legs. This "oversize" is somehow uncouthly embodied in this figure, but instead of being limited to neck and legs it is distributed indiscriminately in all the parts of the animal.

A "wheel-barrow" (Fig. 4)—again from the first patient; the patient succeeded moderately in producing the general shape of a wheel-barrow. Yet the wheel-barrow contains a fundamental mistake: Instead of the wheel it has two more legs and, therefore, lacks the essential feature of a cart.

The same is true for the "hedgehog" (Fig 5.), from a country woman, which was again fairly correct in its general shape. But, having artistically curled hair instead of spines, the patient missed the essential attribute of a hedgehog.

In contrast to these defective aphasic modelings I can show a piece of work

from the aforementioned dentist with psychogenic speech disorder (Fig. 6). This patient eagerly modeled a whole stage setting which is most instructive as projective test to demonstrate his personality troubles, but is far from the defective production of an aphasic.

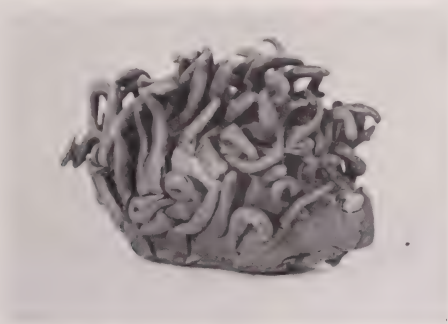


FIG. 5. "Hedgehog."



FIG. 6. "Farm."

These examples which could easily be multiplied reveal the following two essential facts: (1) The linguistic mistakes in aphasia correspond to equal defects in the conceptual pattern of aphasic patients; and (2) These conceptual mistakes arise from defective actualization leading to insufficiently or even wrongly differentiated concepts. This conceptual disorder is still increased by a

severely retarded actualization of concepts which can be demonstrated easily in adequate examinations. Normal language presupposes a quick, as well as, fully differentiated actualization of concepts. Therefore, this disorder must by necessity severely impede the use of language.

On the grounds of this evidence, aphasia seems to be a disorder of conceptual thinking and of concept formation rather than of speech. Admitting only a single aphasic disorder, the conceptual defect *per se* accounts well for the peculiar pattern of symptoms in aphasia namely, the sparing of speech performances which are independent of conceptual thinking (like swearing, conventional or meaningless phrases, etc.), and the impairment of the ability to make a statement (always requiring conceptual thinking) whether made in articulated speech, by gestures, by drawing, or other non-verbal means of expression.

This notion agrees with modern linguistic views about the correlation between language and conceptual order. Yet it excludes any intrinsic relation between aphasia and agnostic type of perceptual disorders or motor defects in the kind of apraxia.

Passing over to this last element of the trinity, apraxia turns out to be a mere produce of speculative imagination created on purely hypothetical grounds which (to use a word of Head) does more to elucidate the ways of productive human thinking than those of the brain function. The hypothetical base of apraxia is the axiom of the strictly symmetrical set-up of nervous functions from a motor and sensory half. Therefore, as paralysis corresponds to anaesthesia, agnosia as a disorder of higher psycho-sensory functions asks for a counterpart on the psychomotor level. This gap in the scheme led to Liepmann's notion of apraxia.

Now, what is actually subsumed under the heading of apraxia is far from being pathogenetically uniform. One set of disorders, e.g., the so-called ideomotor, ideokinetic, limb-kinetic, etc., types of apraxia, are incomplete and therefore somewhat complex sensorimotor disorders of motility, generally in patients with slightly reduced mental capacities. They are quite arbitrarily and artificially separated from the bulk of motor disorders in order to satisfy the theory. Another set of disorders consists of the so-called ideatory or constructional apraxia, apraxia for dressing, etc. Here confusional states, disturbed spatial orientation, and similar general mental disorders account for the patient's failure in complex ideational tasks. Pertinent cases from literature frequently display evident symptoms of aphasia, but these have been de-emphasized in order to save the theory.

As the diverse types of apraxia vary so completely in appearance, there is no practical gain in uniting them under a common denominator. And there is even less reason to postulate a pathogenetical unit which could supply a sensible meaning to the concept of apraxia.

Reviewing the concepts of apraxia, agnosia, and aphasia, in their evolution during the greater part of a century, we find that the rigidly presumed correla-

tion between them was a fiction which severely hampered better understanding of the states themselves. Considered separately, apraxia is a mere hypothetical postulate which represents neither a valid pathogenetic entity nor a useful empirical syndrome. The term apraxia is only apt to create confusion and should be discarded.

Agnosia, in its original meaning, is also derived from untenable theories. Therefore, it does not supply a valid explanation for any pathological state. The term might be used to denominate some clinically similar syndromes. As the resemblance, however, is restricted to mere superficial similarities, we can as well do without this term.

Moreover, the term is dangerous, because its use leads one to believe that we have an ultimate solution to the problems by replacing a clear concept with a melodious Greek word.

In opposition to these phantoms, aphasia is a real pathological entity. Released from the erroneous connexion with apraxia and agnosia it even proves to be uniform in its intrinsic pattern although its clinical appearance may be modified by accidental factors. Unlike the classical views, this specific aphasic disorder does not concern some imaginary processes of verbalization but rather seems to be a disorder of conceptual thinking and of concept formation which affects linguistic processes in so far as language is the most elaborate and most important means for the handling of concepts.

Therefore, we should not waste our time in pursuing the idle notions of agnosia and apraxia, whereas further studies about aphasia promise valuable knowledge of the functioning of the human brain and mind.

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# **"ABORTIVE" LEGG-CALVÉ-PERTHES DISEASE OR DEVELOPMENTAL VARIATION IN EPIPHYSEOGENESIS OF THE UPPER FEMUR**

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While supervising the treatment of children with unilateral Legg-Calvé-Perthes disease at the Blythedale Children's Hospital, minimal surface contour indentations and irregularities were noted in the radiographs of the opposite or apparently uninvolved hip.

There were two references in the orthopedic literature suggestively similar to this finding. Goff referred (1) briefly to this condition, stating that it had "few if any clinical signs to indicate a breakdown" and that it was usually "picked up incidentally on check-up x-ray." He also said that it tended to heal before the first hip. Sundt, in his monograph (2), described 3 cases, all in boys, of mild radiographic changes in the "other clinically healthy hip" while treating clinically unilateral L-C-P disease. The changes regressed after "some months . . . either entirely or to an essential degree." He coined the term "abortive" Legg-Calvé-Perthes disease for this category.

The material analyzed in this paper was extracted from a group of 208 children with Legg-Calvé-Perthes disease: (Fig. 1) 174 males (84%) and 34 females (16%). Eighteen of the patients (15 males and 3 females) had classical bilateral involvement (9%) and conformed to the average statistical incidence of bilaterality. Of the 190 patients remaining, 144 (69%) had classical unilateral involvement accompanied with symptoms of pain and/or limp and signs of varying hip joint restriction all consistent with accepted L-C-P disease picture. Thirty-three (16%) had classical unilateral involvement with the symptoms, signs and radiographic changes ordinarily present in support of the diagnosis of L-C-P but *in addition* showed surface contour changes in the *opposite hip*. These second hip radiographic findings were incidental and *not* associated with clinically demonstrable signs *nor* of history of prior symptoms.

It is to this latter group of 33 cases and its second hip changes that attention is directed. Thirty-one of the children were male and 2 female. The most common pattern of change in the opposite hip was represented in 19 patients by a single, fairly centrally located oval or V-shaped surface defect (Fig. 2). It varied from one to several millimeters in depth (Fig. 3). Occasionally, it could be seen best only in a single view, either the antero-posterior or lateral (Fig. 4). At times, it appeared symmetrically in both views. At other times, it was ovoid in one projection but appeared as a cortical flattening in the other.

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## LEGG-CALVÉ-PERTHES DISEASE

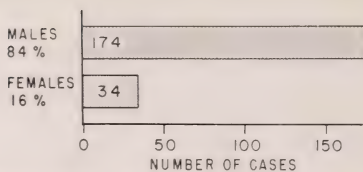


FIG. 1.



FIG. 2. The minimal defect in the left hip is seen best in the A-P view. It consists of a tiny surface contour indentation. It remained for almost two years.

The next most common deviation in the opposite hip, seen in 8 cases, assumed the form of several tiny cortical defects appearing at the same time and producing a scalloped surface configuration (Fig. 5). This usually was seen better in one projection (Fig. 6).

The third segment comprised the remaining 6 cases and represented heterog-



FIG. 3. The minimal involvement seen in the right femoral epiphysis is a deeper V-shaped defect. It is central in location in the A-P view. In the lateral projection its appearance is that of cortical flattening. This lesion lasted almost three years.

enous surface defects. These were noted to be present eccentrically and in portions of the femoral epiphysis other than the usual central or slightly lateral localization.

The single or multiple indentations were occasionally accompanied with some overall trabecular irregularity. Occasionally, mild irregularity occurred along the width of the epiphyseal cartilage plate but this seemed never to be followed by growth retardation or broadening of the femoral head or femoral neck.

Figure 7 shows the distribution of *all* the patients with Legg-Calvé-Perthes disease according to age. The average age for males and females was 6 years with a span covering ages 2 to 14 years. Of the 33 cases (Fig. 8) in the group pertinent to this study, the average age was 4½ years with a span extending



FIG. 4. The minimal lesion in the right femoral epiphysis is centrally located in the A-P view. It is seen less well in the lateral view where it appears as a shallow subcortical rarefaction. The epiphyseal cartilage plate is irregular. The defect lasted for about 17 months.

from 2 to 8 years. The younger average age in the children with minimal defects is noteworthy.

The cases in which minimal defects were found in the opposite hip occurred in males in higher proportion than did the usual form of L-C-P disease. The overall incidence in L-C-P was 84% males and 16% females, whereas in the

group with second hip minimal changes, 94% were males. Only as additional experiences add to the numbers of such patients, can it be determined whether the above associations are constant and reliable.

The length of time during which the minimal contour defect persisted exhibited great variation. The shortest period was 3 months and the longest 38



FIG. 5. In this patient, the wavy scalloped subcortical rarefaction is seen best in the A-P view, laterally. It is not wholly central and tends to spread. In the lateral view, however, the defect appears as a central subcortical rarefaction. The process did not disappear for 29 months.

months. The contralateral hip surface change always disappeared before the classical ipsilateral hip fully recovered. Occasionally, it remained throughout most of the cycle of healing of the more involved side.

The appearance of the minimally involved hip at termination of healing was virtually complete restitution (Fig. 9). This striking feature stood out in bold relief in comparison with the end stage of classical Legg-Calvé-Perthes disease, in which varying degrees of deformity was the rule (3).

## DISCUSSION

The concept of an "abortive" Legg-Calvé-Perthes is attractive. The minimal contour changes seen in the "normal" hip in co-existence with the more severe disorganizations and transformations in the abnormal hip suggested the possi-



FIG. 6. In this patient, the wavy scalloped subcortical rarefaction is seen best in the lateral view of the right hip. In the A-P view the defect appears oval and to be located in the lateral portion of the femoral epiphysis. The process lasted 14 months.

bilities of two varieties of L-C-P. Some indirect evidence was afforded by the reports of Goff and Sundt, already mentioned, of similar experiences in a small number of Perthes patients.

Nevertheless, when this state of affairs was described and the interpretation of two varieties of L-C-P offered, much criticism was provoked. It was posed that the minimal irregularities in the contour of the subchondral bone could also be variants of normal growth. Professional men of great experience who believed these minimal defects to be simple variations in the normal development



stated that it could be seen very often in any subchondral location of growing bone. Caffey's (4) statement was quoted that "when focal irregularities in density are found in the bones of asymptomatic children, the diagnosis of aseptic necrosis should be made with caution."

TOTAL NUMBER OF PATIENTS  
ALL FORMS L-C-P

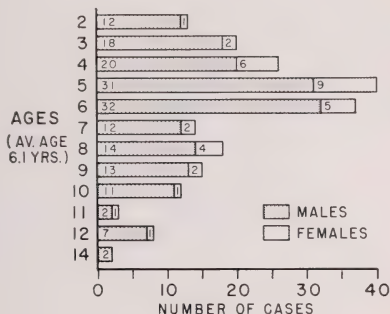


FIG. 7.

CLASSICAL UNILATERAL L-C-P  
MINIMAL CONTRALATERAL HIP

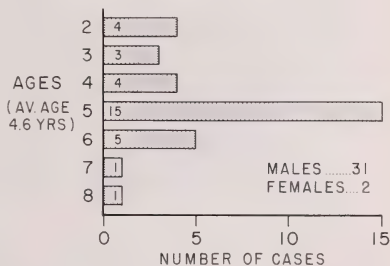


FIG. 8.

A closer examination of the literature revealed much less corroboration of this normal growth variant proposition. Within Caffey's textbook listing the sites of usual irregular mineralization in the growing skeleton, the upper femoral epiphysis was excluded. The humeral trochlea, tibial process and patella were characteristically irregular thruout the period of growth. Irregularities in marginal mineralization in the distal femoral (5) and proximal tibial epiphyses are

limited to certain age levels, as for example, 2 to 6 years in the case of the distal femoral epiphysis.

Burman and Clark (6) speaking of the hip joint of the infant in the first twelve months of life, stated that the growth of the upper femoral epiphysis



FIG. 9. The serial studies of the natural lesion in the right femoral head show the progression towards excellent reconstruction. Characteristically, it heals before the side of major involvement although it may take in excess of two years to do it.

is regular and its trabeculation smooth and even and that at six months of age the epiphysis has attained constancy of appearance.

Martin (7) summarizing the general characteristics of a child's hips, stated that the femoral head epiphyses are present and equal by the age of 3 years and are hemispherical in shape with a horizontal epiphysal line.

# SUMMARY

1. Surface contour irregularities have been described in the contralateral or so-called "normal" hip in 33 cases of classical unilateral L-C-P patients.

2. In this group of patients there were no signs or symptoms relative to the contralateral hip joint changes and discovery was incidental to the bilateral radiographs taken to follow the course of the classical unilateral L-C-P disease.

3. The average age of this group was lower and the male sex incidence higher than in the usual averages of L-C-P disease.

4. Recovery from or disappearance of the surface irregularities took a variable period from several months to years but seemed to result in almost perfect restitution of the form of the femoral head.

5. It is not clear whether the minimal surface contour alterations described are a form of "abortive" Legg-Calvé-Perthes disease or simply a variation in upper femoral epiphyseogenesis. However, since documentation of such growth variation has not been discovered in the literature despite epiphyseal longitudinal growth studies, I am suggesting *abortive* Legg-Calvé-Perthes as an explanation.

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# ISOLATED TRANSECTION OF THE PANCREAS CAUSED BY BLUNT THORACIC TRAUMA

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The frequency of pancreatic transection by blunt trauma to the abdomen is well recognized, but its occurrence as an isolated phenomenon has been infrequently reported since it was first noted by Jaun (1) in 1856. Blandy, Hamblen and Kerr (2), in 1959, summarized the twenty previously reported cases and added one of their own.

The purpose of this paper is to report what is believed to be the first recorded case of isolated transection of the pancreas by blunt trauma to the thorax.

## CASE REPORT

The patient, a 30 year old female, was admitted to The Greenpoint Hospital Emergency Room at 12:55 A.M. on 8, 25 63, complaining of abdominal pain. Thirty minutes earlier, while riding in the right front seat of an automobile traveling at low speed, she was thrown against the dashboard when the driver of the vehicle brought it to a sudden stop, striking her left lower thorax. She was brought to the hospital when abdominal pain developed soon thereafter.

Examination disclosed that the blood pressure was 80/60 and the pulse, 90/minute. The temperature was normal. A recent ecchymosis was noted just beneath the left inframammary fold, but there was no other external evidence of injury. The lungs were clear. The abdomen was scaphoid, but tender in all quadrants; rebound tenderness was present in the left hypochondrium. Bowel sounds were diminished. Pelvic examination revealed marked tenderness upon motion of the cervix; no mass could be felt.

Hemoglobin was 9.0 gm%. White blood cell count was 28,950/mm<sup>3</sup> (3). Films of the chest and abdomen and intravenous pyelogram were normal.

With the infusion of normal saline, blood pressure rose to 110/70, but the pulse remained elevated. Abdominal paracentesis, performed in the right lower quadrant, yielded several cc of blood which failed to clot.

Laparotomy was performed five hours after the injury, with the presumptive diagnosis of ruptured spleen. A left rectus-splitting incision was made, and 250 cc of liquid blood was removed from the peritoneal cavity. The spleen was found intact, but inspection of the pancreas after division of the gastrocolic omentum revealed a sagittal transection 5.5 cm from the tip of the tail. The splenic vessels had been bared opposite the laceration, but were undamaged. A small hematoma

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surrounded the laceration; there was no active bleeding. The retroperitoneal portion of the duodenum was intact, and aside from a 3 cm diameter serous cyst of the right ovary, the remainder of the abdominal organs were found to be normal.

The avulsed fragment of pancreas was removed, together with the spleen. After wedge excision of the distal 1 cm of hemorrhagic tissue from the stump of remaining pancreas, repair was accomplished by suture ligation of the main pancreatic duct and approximation of the anterior and posterior surfaces of the gland with 00 silk. The pancreatic bed was drained with a large-bore sump and a latex tube drain. The abdomen was closed in layers with wire sutures. 1500 cc of blood was administered during the operation.

Postoperatively, all oral intake was withheld and nasogastric suction was maintained for seven days; thereafter, a full diet was gradually resumed. Penicillin was administered for seven days. Temperature remained between 100°F and 101°F for nine days and then returned to normal. Drainage from the abdomen was less than 5 cc daily after 150 cc of bloody serum was obtained during the first twelve hours after operation. On the second postoperative day, analysis of the drainage for amylase activity showed 1,992 King-Armstrong units cc. Serial serum amylase determinations, beginning immediately after operation, were within normal limits, although for the first 36 hours levels of approximately 260 units cc were present. Subsequent values were below 90 units cc.

An asymptomatic left pleural effusion developed, presumably because of contiguous retroperitoneal injury. Removal of the drains was delayed because of continued scant drainage. Skin excoriation around the drains did not develop, and contrast study of the tract on the thirtieth postoperative day failed to demonstrate a collection. After removal of the drains on the thirty-third day, the tract closed rapidly. Secretin test, performed on the thirty-sixth day, and serial blood glucose determinations were normal. The patient was discharged on the thirty-seventh postoperative day in satisfactory condition, although a small left pleural effusion remained. Follow-up films showed its disappearance a month later, and she has remained well since then.

#### DISCUSSION

The mechanism of pancreatic injury by direct blunt abdominal trauma is generally accepted to be that of crushing by direct force against the spine. Less commonly, contre-coup force may be responsible (3). The authors share the discontent of Blandy, Hamblen and Kerr (2) that these are the only possible explanations, especially since the responsible trauma often seems mild when compared to the extent of the injury. In the case reported above, wherein the force was directed obliquely, and to the thorax rather than the abdomen, and where the injury to the pancreas was at some distance from the portion overlying the spine, another mechanism must be supposed. It is our opinion that the sudden deceleration in this case generated sufficient shearing force to cause the injury; this would be analogous to the familiar whiplash injury of the neck.

It must be assumed that a combination of oblique force and anatomic fixation account in some fashion for this particular injury here, rather than a more proximal pancreatic laceration or avulsion of the spleen from its pedicle.

It is generally acknowledged that resection of the portion of the pancreas which lies to the left of the superior mesenteric vessels can be accomplished with a negligible incidence of pancreatic endocrine or exocrine deficiency. Likewise, attempts to preserve the distal portion of a divided pancreas risk a high incidence of fistula, pseudocyst, abscess and hemorrhage, whether direct suture of the gland is attempted or the distal portion is anastomosed to the gut. Therefore, it is apparent that the treatment of choice is removal of the distal fragment of the divided pancreas (4-6). Of the five cases noted by Blandy, Hamblen and Kerr which were so treated, four survived without complication, the fifth dying of secondary hemorrhage.

In the management of patients who have undergone operation upon the pancreas, stimulation of pancreatic secretion is to be avoided until healing is assured. As in the case reported above, it is believed that this measure significantly lessens the risk of pancreatic fistula and its sequelae. Although few drugs at therapeutic dosage have been shown to have a significant depressive effect upon pancreatic secretion, it is certain that endogenous stimulatory factors are markedly reduced when oral intake is withheld and gastric juice is prevented from entering the duodenum.

#### SUMMARY

A case of transection of the pancreas caused by blunt trauma to the thorax is presented. The theoretical mechanism of this injury and the management of distal pancreatic injuries in general are discussed.

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# COMPLICATIONS OF POLYCYSTIC DISEASE OF THE LIVER

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The classification of benign nonparasitic liver cysts into two distinct groups, solitary cysts and multiple cysts or polycystic disease, appears to be advisable both from pathologic and therapeutic standpoints. Polycystic disease of the liver has been defined as a condition in which the liver is involved by cysts of varying sizes. It is generally felt that multiple cysts and polycystic disease represent variants of the same entity. The cysts arise from groups of intra-hepatic bile ducts which fail to involute (1). Histologically they are generally lined by a single layer of cuboidal or low columnar epithelium. The contents are usually amorphous, although occasionally the cyst wall consists of fibrous tissue (2). Of major clinical significance is the frequent association of polycystic liver disease with cystic disease involving other organs (1). The condition may be familial (3). Several extensive reviews of multiple cysts of the liver have appeared in recent years (1, 2, 4, 5). Records are now available in the literature of approximately 380 cases with a suggested surgical incidence of about 0.02% (2).

Instances of polycystic disease of the liver presenting with associated complications are rare (4). The following cases are submitted to illustrate two such complications of polycystic liver disease, namely, obstructive jaundice, and spontaneous rupture with hemorrhage.

## CASE 1

J.D. (M.S.H. #53530), a 44 year old housewife, was admitted to The Mount Sinai Hospital in September, 1955, because of generalized pruritis and a right upper quadrant mass of two months' duration. The patient denied jaundice or the use of hepatotoxic agents. Her appetite had been normal during this period although she experienced frequent episodes of eructation, distention and moderate epigastric discomfort after meals. Progressive dyspnea was noted a week prior to admission. The past history revealed an appendectomy and cholecystectomy 15 and 23 years ago, respectively.

On examination, a soft, non-tender smooth mass was palpable below the xiphoid, filling the right upper quadrant. She was not clinically jaundiced. Pertinent laboratory data were a total bilirubin of 1.94 mg%, with a direct of 1.02 mg%; alkaline phosphatase, 47.8 King-Armstrong units; cephalin flocculation negative; and blood urea nitrogen of 8 mg%. The galactose tolerance test showed some decrease of liver function. Barium meal showed an impression on the posterior surface of the stomach suggesting a retrogastric mass possibly arising from the liver.

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Laparotomy disclosed multiple liver cysts. The largest was 15 cm in diameter. The latter cyst extended from the porta hepatis and contiguous right lobe into the left lobe of the liver. The common and hepatic bile ducts were compressed by this cyst, the ducts from the common hepatic duct proximally seeming to enter into the posterior cyst wall. To the right of this cyst was a 7.5 cm diameter cyst, and two smaller ones were seen to the left of it. The cysts contained colorless, slightly turbid fluid. They were all subtotally excised with obvious release of bile duct compression.



Fig. 1. Microscopic section of wall of bile duct cyst of liver with characteristic cuboidal epithelial lining and surrounding collagenous tissue. Hematoxylin and eosin;  $\times 200$  (Case 1).

The pathologic report of the excised specimen was "bile duct cyst of the liver" (Fig. 1).

Post-operatively, the patient did well until April, 1959, when she was readmitted for recurrent pruritis, again associated with a moderate sized abdominal mass. On this admission, the patient was icteric. A soft abdominal mass was again noted in the right upper quadrant, extending to the level of the umbilicus. Laboratory data was consistent with obstructive jaundice. At surgery, it was seen that the major cyst previously excised had recurred. It filled the right subhepatic space, again compressing the porta hepatis, occupying the contiguous right lobe of the liver and most of the left lobe. The superior wall of the extra-hepatic ducts was intimately attached to the inferior cyst wall and could not

be distinguished from the latter. Inasmuch as complete excision could not be done, and recurrence after subtotal excision likely, a Roux-en-y cystenterostomy was performed. In addition a T-Tube was inserted into the common duct.

A liver biopsy showed focal fatty infiltration and moderate bile stasis. A subsequent T-Tube cholangiogram revealed a short normal cystic duct stump.



FIG. 2. T-Tube cholangiogram demonstrating extrinsic pressure on bile ducts by residuum of anastomosed recurrent cyst occupying medial right and most of left lobes of liver (arrows).

The main hepatic radicles appeared separated. There was extrinsic pressure on the right hepatic duct just proximal to its junction with the left. The left duct was straightened and its outermost tributary curved downwards. The branches of the right hepatic radicles near the extrinsic pressure defect appeared to surround a mass. These findings coincided with the operative findings and suggested the residuum of the post anastomotic cyst (Fig. 2).

Since discharge, the patient has been asymptomatic, the last follow-up being December, 1963, eight years after the onset of her first symptoms.

*Comment*

This case is unusual in that sufficient pressure was produced by the major cyst overlying the porta hepatis to cause obstructive jaundice. In addition, to solve the problem of recurrence of the bile duct cyst after subtotal excision, an anastomosis of the cyst to the jejunum was employed to achieve permanent decompression.

## CASE 2

L.K. (M.S.H. #232292), an 80 year old white male, entered The Mount Sinai Hospital in October of 1963 with the sudden onset of right upper quadrant abdominal pain five hours prior to admission. The pain became more generalized and was accompanied by a single episode of vomiting and progressive dyspnea. No prior abdominal or gastrointestinal symptoms were noted. During the year prior to admission there had been increasing abdominal girth.

Physical examination revealed a heavy-set, elderly male appearing dyspneic, dehydrated, and acutely ill. Dullness with diminished breath sounds were heard at both lung bases. The abdomen was protuberant, and diffusely tender, most marked in the right mid-abdomen. There was generalized rebound tenderness. A smooth mass extending to the level of the pelvic brim was palpable on the right side of the abdomen. No stigmata of liver disease were apparent.

Initial laboratory tests disclosed normal values for hematocrit, blood count and differential, urinalysis, blood urea nitrogen, sugar, serum electrolytes and liver function studies. Chest x-ray showed an elevated right diaphragm. Films of the abdomen demonstrated a right-sided mass which displaced the small and large bowel downward and to the left.

After six hours of observation, the abdominal findings persisted and the patient was explored through a right paramedian incision. The peritoneal cavity contained a liter of brownish colored fluid. Serosal reaction to this fluid was minimal. Three large, thick-walled cystic masses originated from the liver surface. At several areas the cyst walls were adherent to the omentum. Large dilated veins traversed these areas covering the cyst. One cyst arising from the left lobe of the liver measured about 35 cm in diameter. The cyst so compressed the surrounding left lobe of the liver that only a thin rim of this lobe could be identified. A second cyst measuring about 20 cm in diameter was situated just to the right of the falciform ligament and was intimately connected with the lower anterior convex surface of the liver. A third cyst occupied the remainder of the right upper abdominal cavity. A 2 cm perforation was located over the anterior aspect of the latter cyst. Leakage of dark greenish-brown material appeared through this opening and fresh blood oozed slowly from the torn edges of the perforation. The bulk of normal liver tissue was displaced high under the diaphragm and posteriorly. The cysts from left to right contained 1800, 320, and 400 cc of fluid. Following decompression of straw colored fluid from the cysts by trocar aspiration, numerous smaller cysts could be seen projecting from the undersurface of the liver (Fig. 3).

A small segment of each major cyst was excised and the interiors explored. The

cysts were filled with yellowish-brown turbid fluid. They were lined by a smooth membrane with occasional loculations. A large soft rubber Bardex tube was inserted into each of the cyst cavities. The cut edges were over-sewn and the

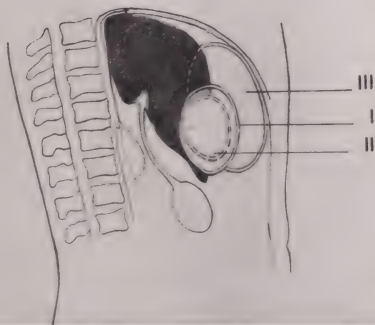


FIG. 3. Case 2—Diagram of operative findings demonstrating location of major giant cysts (I, II and III) and volume of fluid evacuated from each. Cyst I shows point of rupture with leakage of fluid content.

tubes secured in place. A total of three tubes was used, each being brought out through the abdominal wall through a separate stab wound.

The fluid aspirated from each cyst revealed a total protein ranging from 0.2

4.1 mg per cent; albumin 0.4-2.2 mg per cent; globulin 0.4-1.9 mg per cent; amylase 12-22 units; calcium 4.4-7.2 mEq L; and cholesterol 20-75 mg per cent. Because of evidence of chronic pulmonary disease and poor ventilatory exchange, a prophylactic tracheostomy was performed.

On the fourth post-operative day while the tracheostomy was being suctioned, the patient eviscerated. The evisceration was repaired by four No. 5 silk through retention sutures. The remainder of the patient's hospital course was uneventful. Additional studies prior to discharge revealed a cystic lesion of the right renal kidney. This association has been previously reported (1).

### *Comment*

The cystic fluid found in previous reports contained hemic elements, variable protein contents and small amounts of bile pigments (2, 8), as did the case reported above. Microscopic examination of segments of the cyst wall showed a cuboidal to columnar epithelial lining with fibrosis and occasional glandular elements consistent with bile duct cyst.

### DISCUSSION

Polycystic disease of the liver has been noted at all ages, frequently presenting itself after the fourth decade. Most of the cysts remain clinically undetected and cause little or no discomfort (1). The more common symptoms of polycystic liver disease are the direct result of pressure on adjacent viscera produced as the cysts enlarge. In such cases, a mass or fullness, abdominal pain, and non-specific digestive complaints are frequent findings. In a recent series of 29 cases, 12 (41 per cent) of the cases complained of a gradually enlarging mass; 10 (34 per cent) of pain; 4 (14 per cent) of indigestion; and 2 (7 per cent) of dyspnea (5).

Jaundice is a rare complication of multicystic disease of the liver because growth is so slow that adjustment to the increasing pressure is the rule. Autopsy findings have suggested that the jaundice may result not only from pressure on the common duct and hepatic radicles (5, 6) but from disruption of normal hepatic architecture by cyst formation (5). In Case 1, the T-Tube cholangiogram confirmed the extrinsic pressure and distortion of the biliary tree produced by the bile duct cyst. However, the jaundice was due to pressure on the extrahepatic bile ducts. In this case, the superior wall of the hepatic ducts and the contiguous part of the inferior cyst wall seemed to be a common structure. As mentioned before, such cysts are considered to arise from faulty fusion or involution of ductal elements or accessory bile ducts (1, 7). While this has had reference to a process involving the intrahepatic bile ducts, thought should be given to the possibility of origin from the extrahepatic bile ducts as well, as a type of reduplication.

Spontaneous rupture of nonparasitic cysts is rare; only five cases have been mentioned in the literature (8-11). All presented with an acute surgical abdomen. One died in the immediate post-operative period (10).

Laboratory findings in 60-70 per cent of the cases of polycystic liver disease



reveal normal hepatic function (5). Roentgenograms may demonstrate the presence of a space occupying mass in the upper abdomen with displacement of the stomach or colon or elevation of the diaphragm.

The life expectancy of patients with polycystic disease of the liver is affected only in these rare cases of progressive biliary cirrhosis or spontaneous hemorrhage and perforation. Thus in most instances, polycystic disease of the liver by itself does not appreciably affect the health of the patient unless associated with cystic disease of the kidney. The latter association is present in about 50 per cent of the cases of polycystic liver disease.

The treatment of multiple liver cysts is usually not surgical if symptoms are minimal. Surgery is not curative since rarely is the disease limited to a technically resectable portion of liver (4). There are several acceptable surgical approaches. These include simple aspiration; external drainage; marsupialization; subtotal excision; and cystenterostomy. Cystenterostomy, commonly used for pancreatic cyst, has only rarely been employed in the treatment of hepatic cyst.

Recurrence following surgical management can occur, as illustrated by Case 1.

#### SUMMARY

1. Two cases of polycystic disease of the liver complicated by obstructive jaundice and spontaneous rupture and hemorrhage are presented.

2. The case of obstructive jaundice was temporarily relieved by subtotal excision of the cyst only to recur five years later. Roux-en-y cystenterostomy as a secondary procedure has apparently afforded long term relief.

3. The case of spontaneous rupture of a large hepatic cyst was successfully treated by external tube drainage.

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## CLINICO-PATHOLOGICAL CONFERENCE

### Abdominal Pain in an Elderly Man

*Edited by*

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An 82 year old white male was admitted to The Mount Sinai Hospital because of abdominal pain for 7 days. The pain began in the right upper quadrant, shifted to the left and then settled in the left lower quadrant where it continued until admission. The pain was associated with a fever of 101°, anorexia, urinary incontinence and occasional dysuria. There was no history of vomiting, change in bowel habits, melena, hematuria, or weight loss. The patient was a known diabetic for 20 years, controlled with 40 units NPH insulin daily. During the year prior to admission, he had developed gangrenous lesions on both lower extremities which had been treated medically with satisfactory results. He had had chronic constipation for many years, and for the past 1 to 2 years had been unable to walk because of weakness and gangrenous lesions of the lower extremities. A member of his immediate family was diabetic.

The blood pressure was 168/68, the pulse 80 per min with occasional premature contractions, the respirations 14 per min, and the temperature 101°F. He appeared well developed, but chronically ill. He was confused and poorly oriented, complained of pain in the abdomen. There were gangrenous lesions on both feet. No lymph nodes were palpable. Bilateral cataracts and Grade II-III diabetic retinopathy were present. He was edentulous and the neck was supple. Auscultation of the lungs revealed a few diffuse rhonchi bilaterally, and inspiratory rales at the right lung base which cleared with coughing.

The heart rhythm was regular except for an occasional dropped beat.  $A_2$  was greater than  $P_2$ , and a grade III harsh systolic ejection type murmur was heard at the apex and along the left sternal border and at the aortic area. The abdomen was soft and there was no rebound tenderness. Increased bowel sounds were heard and there was tenderness to palpation over the entire abdomen. A 4 x 6 cm mass which was very tender, regular and firm, was felt deep in the left lower quadrant adjacent to the midline. The liver and spleen were not felt. There was neither costovertebral angle tenderness nor edema. Rectal examination revealed an enlarged prostate. Dorsalis pedis and posterior tibial pulses were absent bilaterally and ankle and patellar reflexes could not be elicited.

The urine contained moderate protein, but no acetone nor glucose. The urinary sediment had occasional red blood cells and 1-2 white blood cells per high power field. The hemoglobulin was 10.6 G%, the white blood cell count 20,000  $\text{mm}^3$  with 78% segmented cells, 13% band forms, 1% lymphocytes, and 8% monocytes. The sedimentation rate was 115 mm per hour. The blood glucose was 116  $\text{mg}\%$ .

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the blood urea nitrogen 24 mg%, the serum sodium 127 mEq L, potassium 3.3mEq L,  $\text{CO}_2$  27.4 mEq L, and chloride 96 mEq L. The total serum cholesterol was 190 mg%. Stool guaiac examinations were 3+ and 0 on two occasions. The blood and urine were sterile. The chest x-ray was normal. Abdominal x-rays showed distention of the small bowel and a moderate amount of gas in the large bowel and stomach. No definite mass was identified. An intravenous pyelogram was normal except for an enlarged prostate as was the barium enema.

Electrocardiogram showed a normal sinus rhythm with frequent atrial premature systoles. The P wave was wide and notched, a small r and deep S was present in  $V_1$ . There was a Q wave in  $V_2$ , ST segment depression in  $V_1$ - $V_6$ , and T waves of low voltage in leads I and AVL.

In the hospital, his temperature fluctuated between 99° and 102°. His abdominal pain gradually decreased and the left lower quadrant mass was no longer felt. However, his condition deteriorated and he became semistuporous, requiring tube feeding. On the thirty-fourth hospital day diffuse rhonchi were noted. He became cyanotic and unresponsive, and died on the forty-first hospital day.

*Dr. E. Marvin Sokol:*\* We are dealing with an 82 year old diabetic man who came to the hospital after a week's history of abdominal pain. The pain was interesting in that it began in the right upper quadrant, migrated to the left and finally settled in the left lower quadrant. This suggests a gastrointestinal disorder. We are not told, however, whether the pain was intermittent or colicky in nature. It was accompanied by fever and the patient was anorectic.

Attention is also directed to the genitourinary system because of incontinence and dysuria. The patient had a large prostate, but his urine culture and intravenous pyelogram were unremarkable.

The most important features of the physical examination were limited to the abdomen. There were diffuse abdominal tenderness and hyperactive bowel sounds. Located in the left lower quadrant was a mass, which was four by six centimeters in size and located near the midline. It was regular and firm, but no mention is made of a bruit or if it was movable. This mass subsequently disappeared.

The other important physical findings involve the cardiovascular system. There was no evidence of congestive failure, but the patient did have a systolic murmur, which was ejection in type and heard along the left sternal border, the apex and the aortic area. I believe the murmur was that of calcific aortic stenosis.

The laboratory examinations were helpful in some respects. A white count of 20,000 with a shift to the left, and an elevated sedimentation rate suggest an inflammatory process. The patient was slightly anemic, but we have no reason to suspect that there was significant active gastrointestinal bleeding since one stool examination was guaiac negative. The blood urea nitrogen was modestly elevated, and the serum sodium and chloride concentrations were depressed.

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In the absence of edema, I do not think this was dilutional but rather sequestration within the peritoneal cavity or the intestinal tract. I have asked Dr. Goldman of the Department of Radiology to review the x-ray findings.

*Dr. Richard Goldman:*\* The admission chest film showed no evidence of active pathology in either lung. Unfortunately, the patient was rotated and it was difficult to evaluate the heart size but there was a slight elevation of the right diaphragm.

An intravenous urogram done four days following admission showed no evidence of pathology of either collecting system. There was a smoothly nodulated

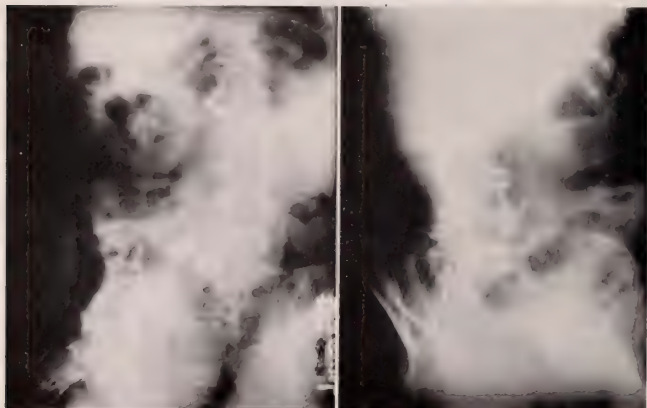


FIG. 1. Scout film of abdomen showing moderate dilatation of loops of the small bowel.

FIG. 2. Oblique film of the abdomen. Arrow indicates mottled collection of gas.

mass markedly elevating the base of the bladder, consistent with an enlarged prostate gland.

A barium enema examination done nine days following admission showed no evidence of intrinsic colonic pathology. The initial scout film of the abdomen showed moderate dilatation of some loops of small bowel. There was air and fecal material seen throughout the colon, which was not dilated. Air was present in the rectum. Sequential films taken in the first week of hospitalization showed greater dilatation of loops of small bowel (Fig. 1), and dilatation of the small bowel was also seen on the barium enema examination. This pattern would suggest an adynamic ileus. Because of the dilatation of the small bowel and relative absence of air in the colon, an obstruction cannot be entirely excluded. Perhaps of greater significance is a peculiar mottled collection of gas located

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anteriorly in the left lower quadrant and present on every film (Fig. 2). This could represent an abscess, necrotic tumor mass, or an acute infarction of the small bowel.

*Dr. Sokol:* Is there any evidence of air in the biliary tree or calcifications of the mesenteric vessels or in the heart?

*Dr. Goldman:* There is no evidence of air in the biliary tree; some linear calcifications were noted in the region of the hypogastric vessels. I did not see any calcification within the abdomen or heart shadow, nor did I see evidence of a foreign body.

*Dr. Sokol:* We can summarize the positive x-ray findings by stating that there appeared to be a paralytic ileus or an incomplete obstruction. I favor incomplete obstruction because there were hyperactive bowel sounds. The x-rays are not consistent with a volvulus or an internal hernia as a cause.

The most important x-ray finding is the abscess-like cavity in the left lower quadrant. I think that this represents a gangrenous or perforated small bowel segment, secondary to vascular compromise, and could result from either primary vascular occlusion or a strangulated loop.

Another condition that has to be considered is a foreign body. An elderly, confused man could have easily swallowed a fish bone which could have perforated the small bowel. Necrosis of a small bowel tumor has been mentioned. A perforated viscus, such as an appendix, or a duodenal ulcer can also result in an intra-abdominal abscess. However, I do not think that it would localize in the left lower quadrant. Diverticulitis of the colon has been ruled out by the barium enema.

The electrocardiogram showed evidence of an old anterior myocardial infarction. I believe this was due to arteriosclerosis since we have additional evidence of peripheral vascular insufficiency. An interesting finding is the widening and notching of the P waves. These are usually seen in mitral valve disease, however, the murmur was not typical. I think this patient's P-wave changes were secondary to fibrosis of the atrium with delay in depolarization. This P-wave change has also been described in atrial tumors, such as a large atrial myxoma.

Because of his semistuporous condition, nutrition was inadequate and tube feeding was required. Under these circumstances aspiration pneumonia is common.

I shall confine the remainder of my discussion to the abnormality in the left lower quadrant. I think intrinsic colonic or genitourinary pathology has been ruled out. For this to be an aneurysm of a large abdominal vessel, I would have to postulate that it ruptured since the mass disappeared. If it had ruptured, the patient certainly would have had a massive intraperitoneal hemorrhage. Therefore, I think the small bowel was the primary site of pathology. I asked Dr. Goldman specifically about air in the biliary tree because when I read the protocol for the first time, the migration of the pain, beginning in the right upper quadrant and localizing in the left lower quadrant, certainly suggested a gallstone ileus. However, there was no air in the biliary tree.

Intussusception and vascular occlusion of the small bowel could account for

obstruction, abdominal pain, a mass, fever, leukocytosis, and finally perforation. Intussusception is a disease of the young, even though it has been reported in older people. The symptoms are usually described as paroxysmal and crampy pain, vomiting and the passage of bloody mucous. None of these findings were described in the present patient. In this case the presence of an abdominal mass which disappeared suggested a diagnosis of intussusception, since a tumor is palpated in 30 to 60 per cent of adults. An x-ray of the small bowel frequently demonstrates the lesion, and occasionally the intussusceptum can be seen as a mass within an air fluid cavity on routine abdominal films. I would have to postulate an enterocenteric intussusception since the barium examination was normal even though enteric intussusceptions are much less common than the ileocolic or colocolic types. In adults, intussusceptions are secondary to some other small bowel pathology in almost 90 per cent of the cases. Although benign tumors such as adenomas, lipomas, fibromas, and myomas are a frequent cause, it can also be produced by carcinoma, lymphoma, or melanoma. Meckel's diverticula also account for a significant number both in adults and children.

Mesenteric vascular occlusions usually result in intestinal infarction but there may be infarction without an organic occlusion. The extent of a mesenteric infarction depends on the area of occlusion, the occluded vessel and the patency of the collateral vasculature. Infarctions of the small bowel both venous and arterial are almost always hemorrhagic in nature. This is easily explained in venous occlusions since there is a huge backing up of blood resulting in an engorged fluid filled loop of intestine. In arterial occlusions it is felt that the blood ingresses via retrograde venous flow. Sepsis, trauma and hypercoagulable states such as polycythemia vera are known predisposing causes. None of these were present in this case. Distention of the intestinal tract may also lead to venous and eventually arterial occlusion. In addition, venous compression, particularly by tumor; or a portal vein thrombosis may lead to mesenteric thrombosis. Finally, thromboangiitis obliterans involving the abdominal vessels can produce both arterial and venous occlusions.

Arterial occlusions account for the majority of mesenteric infarctions, but differentiation between arterial and venous may be difficult. Venous occlusions usually have a more insidious onset and are accompanied by more profuse intestinal bleeding than occurred in the present case. The three main nutrient vessels which arise from the abdominal aorta are the superior mesenteric, the inferior mesenteric and the celiac arteries. The superior mesenteric artery is usually occluded, both by thrombosis and embolization, because of its peculiar anatomic location. The usual cause of thrombosis of this vessel is arteriosclerosis, and this patient had evidence of diffuse arteriosclerotic involvement including the peripheral and coronary vessels. However, in most cases of superior mesenteric thrombosis, secondary to arteriosclerosis, there is usually a prior history of gastrointestinal symptoms. Classically, this is intestinal angina and occurs when other vessels of the mesentery are affected. A much less common cause for thrombosis is spasm, secondary to shock or congestive heart failure. A dissecting



or local arteriosclerotic aneurysm may occlude an orifice of a mesenteric vessel and result in thrombosis.

No clinical pathological conference is complete without mentioning periarteritis nodosa and lupus erythematosus, since both can give small areas of mesenteric infarction. We have no evidence of such a systemic illness in this case.

The last category of arterio-mesenteric vascular occlusions is a result of embolization—most commonly from a ventricular thrombus secondary to arteriosclerotic heart disease and myocardial infarction. Bacterial thrombi or an aortic atheroma may also embolize to a mesenteric vessel. Lastly, I have to consider atrial myxoma although it is very rare.

In a case report which appeared in the *British Heart Journal*,\* an elderly diabetic woman was described who presented with a massive mesenteric occlusion having embolized from an atrial myxoma. It was also interesting to me that her electrocardiogram was almost identical to the present case.

In summary, I think the patient suffered from an infarction of a portion of the small bowel. This infarction resulted from an occlusion of an already compromised superior mesenteric artery or major tributary. The embolus came from the left side of the heart, and the patient's demise was hastened by an aspiration pneumonia. I believe the patient also had diabetes, cerebral, coronary, and peripheral arteriosclerosis.

*Dr. Stern:*† At autopsy there was 100 ml of yellow fluid in the right pleural cavity. The right lung weighed 680 g and the left lung weighed 480 g (the upper limit of normal about 400 g). The lung parenchyma was hypo-aerated, and there were patches of grayish consolidation scattered throughout both lungs. Microscopically, the alveoli were filled with polymorphonuclear leukocytes, as well as vegetable and meat fibers surrounded by foreign body giant cells. Since a well developed foreign body giant cell reaction was present, the process must have been present for several days prior to his demise.

The serous membranes of the abdomen were clear and transparent, except for an area in the left lower quadrant where fibrinous and fibrous adhesions plastered a small segment of small bowel to the peritoneum of the anterior abdominal wall. On opening the bowel, a sharply demarcated lesion was found. The mucosa was absent over this area and the bowel wall was thin and friable (Fig. 3). Microscopically, muscle fibers were absent in the involved portion of bowel and the wall of the bowel was composed of connective tissue and inflammatory cells (Fig. 4). This lesion is characteristic of infarction of the small intestine. Over the last 13 years, we have had three other similar cases. Their history and the pathologic findings were similar to this case.

Dr. Oh of the Department of Surgery tied off distal small branches of the superior mesenteric artery in several rats. When sacrificed two weeks later no changes were found in the small intestine. Therefore experimental occlusion of

\* Guthrie, T. Aortic Embolization. *Brit. Heart J.*, 25: 137-40, 1963.

† Former Resident in Pathology. The Mount Sinai Hospital, New York, N. Y.

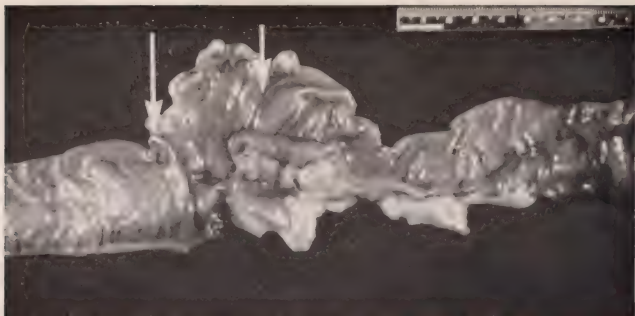


FIG. 3. Infarcted segment of ileum (arrows). The involved segment is kinked by serosal adhesions.

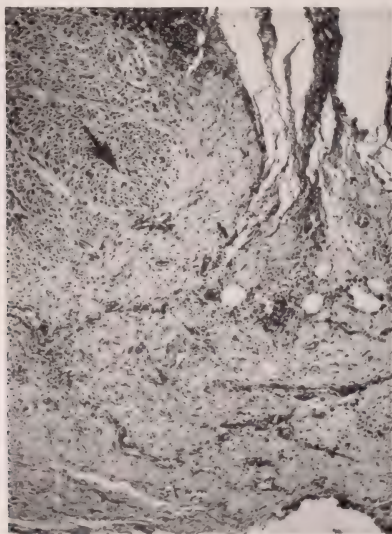


FIG. 4. Photomicrograph of involved ileum (H & E  $\times 400$ ). Muscularis of adjacent area (arrow) abruptly disappears. The infarcted area is thinned and composed of connective and granulation tissue.

a branch of the superior mesenteric artery produces no changes, whereas an occlusion of the main trunk produces massive necrosis. I believe the segmental infarction in our case is the result of an acute occlusion of a branch of the superior mesenteric artery, superimposed upon severe arterosclerosis which limited the anastomotic arterial supply.

The spleen also showed a large infarct and suggested that both lesions resulted from embolization. The splenic infarct was old and contained acellular fibrous tissue showing marked calcium incrustation and iron pigment.

The heart weighed 300 Grams and attached to the endocardium of the left atrium was a five centimeter long soft, red, gelatinous mass. Microscopically, this mass had many finger-like projections. It consisted of a rather pink homogeneous matrix with a fairly sparse cellular population consisting of round, stellate and some multinucleated cells. In some areas, there was an endothelial-like covering. Attached was a thrombus. At its point of attachment to the endocardium there was ingrowth of new vessels, hemorrhages and abundant iron pigment.

Whether all atrial myxomas are true neoplasms or are all organized thrombi is as yet undecided. Current evidence favors a true neoplastic origin. The electron microscopic studies have not demonstrated collagen fibers in atrial myxomata, whereas they are always present in organized thrombi. In addition, cardiac myxomas are almost always in the atrium, whereas thrombi are usually in the ventricles. Atrial myxomas also contain acid mucopolysaccharides, which are absent in organized thrombi. Acid mucopolysaccharide was demonstrated in the present case. In favor of a thrombotic origin is the abundant iron pigment, the ingrowth of blood vessels at the base, and the areas of fibrin deposition. The present case showed abundant fibrin deposition.

Finally, the kidney showed the characteristic lesions of intercapillary nodular glomerulosclerosis as well as a diffuse thickening of the basement membranes consistent with diffuse glomerulosclerosis.

**FINAL DIAGNOSIS:** 1) Segmental infarction of small intestine, secondary to thrombus from: 2) left atrial myxoma, 3) generalized atherosclerosis, 4) diabetic glomerulosclerosis.

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## RADIOLOGICAL NOTES

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*New York, N. Y.*

CASE NO. 254

This infant girl was born to a diabetic mother who had toxemia in the last trimester and was delivered by Cesarean section. There was no evidence of respiratory distress after the delivery. At one day of age, the baby was noted to have a distended abdomen and had frequent bouts of vomiting greenish material. No meconium was passed.

Examination revealed an acutely ill baby who was slightly dehydrated. The abdomen was distended and a questionable, slightly tender soft mass was felt in the right upper quadrant.

Laboratory examination revealed a hemoglobin of 14.6 Gm, a white cell count of 15,000 with a normal differential, and a hematocrit of 34 per cent. Blood electrolyte studies were within normal limits.

Radiographic examination of the abdomen in the supine projection revealed the gas-filled stomach to be normal in size and position. Considerably distended loops of small intestine were seen in the left side of the abdomen. No gas-filled loops of intestine were noted in the right side of the abdomen. There were no abnormal calcifications (Fig. 1A).

Barium enema was then performed which revealed a normal rectum and recto-sigmoid. The colon down to the distal sigmoid was markedly narrowed in a generalized fashion as seen in microcolon. There were a few filling defects in the descending colon and sigmoid which appeared to represent meconium plugs. There was an associated malrotation of the cecum which was noted to be in the right upper quadrant. The distended loops of small intestine were again seen in the left upper quadrant (Fig. 1B and 1C).

Laparotomy was then performed, at which time an ileal atresia was found 15 cms proximal to the ileo-cecal valve. The small bowel was grossly distended proximally. The cecum was noted to be in the right upper quadrant. In addition, there was a volvulus of the small intestine around a dense fibrotic band extending across the atresia towards the gallbladder. The color of the small bowel for a distance of 6 cms proximal to the atresia was deeply purple and deemed non-viable. An ileocolic resection was performed with an end-to-end anastomosis. The baby had an uneventful recovery.

### DISCUSSION

In patients with ileal atresias, symptoms of intestinal obstruction occur during the first day of life and are always progressive. The use of a barium enema, as in the case presented, is helpful in ruling out colonic abnormalities and other associated congenital anomalies. Six per cent of small bowel atresias have co-existing congenital malformations of the gastro-intestinal tract, such as mal-

From the Department of Radiology, The Mount Sinai Hospital, New York, N.Y.



Case 254, Fig. 1A. Supine examination of the abdomen reveals a normal gas-filled stomach. Moderately dilated loops of small intestine are noted in the left side of the abdomen. There is an absence of bowel loops on the right side. No soft tissue masses or calcifications are seen.



Case 254, Fig. 1B. Barium enema reveals the caliber of the colon down to the sigmoid to be markedly narrowed as seen in microcolon. Within the sigmoid and descending colon, there are a number of filling defects which have the appearance of meconium plugs. The cecum is malrotated and presents in the right upper quadrant. Dilated loops of small bowel are again seen in the left upper quadrant.



rotation (1). In the case presented, the barium enema demonstrated the micro-colon typically seen in small bowel atresias and a few plugs of meconium can be delineated within the descending colon. The presence of the malrotation was



Case 254, Fig. 1C. In the lateral projection, no further abnormalities are noted.

also demonstrated by means of a barium enema, although this was suspected from the absence of intestinal contents in the right side of the abdomen on preliminary films.

*Case Report:* ILEAL ATRESIA WITH MALROTATION AND VOLVULUS.

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## CASE NO. 255

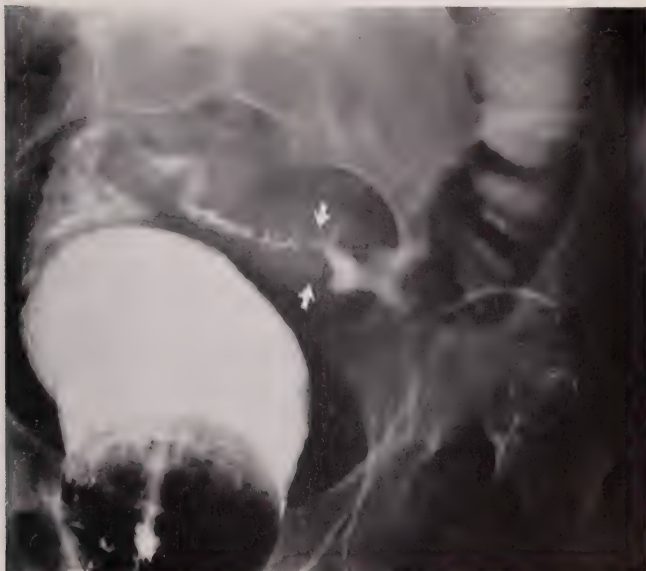
A 72 year old female was admitted to the hospital because of ten days of profuse watery, foul-smelling diarrhea.



Case 255, Fig. 1. Preliminary film prior to barium enema shows a long thin streak of air (arrow) corresponding to the sigmoid, which joins more normally distended bowel proximally and distally.

The patient was hospitalized fifteen months previously for a mild right sided hemiparesis. A prior history was elicited of heart disease for 35 years. Auricular fibrillation had been present on one occasion which reverted to regular sinus rhythm on digitalis therapy. Physical examination revealed regular sinus rhythm, cardiac enlargement, and an apical presystolic murmur. The patient also complained of coldness and lethargy of long duration, and had a dry skin, thick speech, coarse voice and periorbital edema. Protein bound iodine determination was  $3.5 \mu\text{g}\%$ . The diagnoses were rheumatic heart disease, myxedema, and mild cerebro-vascular accident. The patient was treated with digitalis and thyroid extract. The symptoms of myxedema and the mild right hemiparesis rapidly

disappeared and she was discharged to a nursing home. One year later, the patient was again admitted with a right hemiparesis, this time severe. Aphasia was also present. The patient recovered after a stormy course but considerable neurologic deficit remained. She was again discharged to a nursing home. Ten days before admission the patient experienced a sudden episode of profound



Case 255, Fig. 2. Barium study of the same area shows marked narrowing of the sigmoid with a hazy, irregular surface pattern. The serosal contours are well-defined (base of upper arrow and mid-portion of lower arrow) revealing a smoothly thickened bowel wall.

shock. This was accompanied by abdominal pain, distention, fever and severe watery, foul-smelling diarrhea. She was treated vigorously for shock and heavy antibiotic coverage was included. On the day of admission dark red blood appeared in the stool, ten days after the initial episode.

Radiographic studies of the abdomen in multiple projections on the day of admission showed colonic distention with considerable gas and fecal matter but no definite pattern of obstruction. Radiographic study of the chest showed a large cardiac silhouette with a straight left contour, a double density seen

through the cardiac silhouette, and some pulmonary congestion. There were also pneumonic infiltrations in the right lower lung field. The patient ran a febrile course and heavy antibiotic coverage was continued. Three days after admission, a barium enema was performed.

Preliminary film of the abdomen prior to barium enema examination (Fig. 1) revealed a thin straight streak of air in the expected location of the sigmoid colon with sharp transition proximally and distally to more normally distended



**Case 255, Fig. 3. Repeat barium study one week later shows a more distensible sigmoid with cobble-stoned surface pattern.**

bowel. With barium filling, a partial obstruction was reached in the sigmoid region (Fig. 2). Only small amounts of barium passed the sigmoid where a 10 cm long narrowed segment was visualized in which the mucosa was hazy and irregular with a pattern of small nodular shadows throughout. The serosal contours of the sigmoid were discerned revealing a smoothly thickened bowel wall. No diverticula were seen, no constricting lesion or shouldered margins were demonstrated, and there was no extravasation of barium. A diagnosis of infarction of the sigmoid due to a vascular accident was advanced.

The patient's clinical condition stabilized and diarrhea improved. Rectal bleeding ceased. A repeat barium enema was performed one week later (Figs. 3

and 4). At that time the sigmoid was somewhat more distensible. A well developed nodular fold pattern was seen throughout the involved segment and barium between the nodular folds created a cobblestone appearance. Again there was no definite evidence of diverticula, tumor or perforation.

Diarrhea improved remarkably and the patient was discharged to a nursing home. However, the patient expired suddenly two weeks later, presumably due



Case 255, Fig. 4. Post-evacuation film gives a better indication of the extent of involvement distally, where the cobble-stoned pattern is exquisitely demonstrated.

to another cerebral vascular accident. Post mortem examination was not obtained.

#### DISCUSSION

In recent years there has been increased awareness of the radiographic features in acute vascular deprivation of the bowel. Whereas in the past interest had centered on massive infarction of the small bowel due to arterial or venous occlusive disease with resultant gangrene, we now must emphasize the condition of segmental infarction of the bowel which does not lead to frank gangrene, but is in fact reversible to various degrees. The etiology is usually arteriosclerotic oc-

clusive disease and, less frequently, arterial embolization. The radiographic features reflect the pathologic process, and initially the involved segment of bowel shows marked edema of the mucosal folds, thickening of the bowel wall and narrowing of the lumen. Although distention of the bowel proximally may be prominent, the obstructive picture can usually be managed conservatively with intubation. Bleeding is almost always present and the bleeding may be massive. Resection is usually not required in the initial phase, providing that frank gangrene does not supervene. As resolution occurs following the acute insult the edematous and thickened mucosal folds lose their hazy quality and become smaller and more sharply outlined. In some instances the bowel eventually returns completely to normal. In others, the mucosa may atrophy leaving a smooth segment of bowel. In more severe cases fibrosis of the bowel wall leads to a smooth stricture. The ultimate result depends on the severity of the initial insult, the development of collateral circulation and whether or not significant bowel wall infection has occurred. Heavy antibiotic therapy is therefore indicated.

In the case presented, a patient with severe rheumatic mitral disease and multiple embolic episodes, it is presumed that a small embolism occluded a sigmoid mesenteric vessel. The radiographic features in this case include markedly nodular edematous mucosa with narrowing of the bowel lumen and thickening of bowel wall (Fig. 2). Some improvement was apparent on the second barium enema study (Fig. 3), but unfortunately no further follow up could be obtained.

The differential diagnosis includes acute diverticulitis, segmental granulomatous colitis, and radiation sigmoiditis. Scirrhou carcinoma intrinsic to the sigmoid and metastatic carcinoma to the sigmoid must also be considered.

*Case Report:* INFARCTION OF THE SIGMOID COLON.

#### ACKNOWLEDGMENT

This case is presented through the courtesy of Dr. Paul Barr, Good Samaritan Hospital, Suffern, N. Y.

#### CASE NO. 256

A 27 year old man was admitted to the hospital because of intermittent episodes of abdominal pain and diarrhea for one month with increasing intensity and severity for four days prior to admission. There was one episode of emesis at the onset of symptoms. There was no bleeding. One similar episode has occurred two years previously which subsided spontaneously. Physical examination revealed a doughy mass in the right lower quadrant which was tender to palpation. There was clubbing of the finger tips.

Gastrointestinal radiographic studies were performed. The esophagus and stomach were normal. The duodenal bulb (Figs. 1 and 2), was uniformly narrowed and the mucosa showed a frayed appearance. No mucosal folds were noted. There was no clear delineation of a pylorus and the gastric antrum and the duodenal bulb merged imperceptibly. There was a sharp transition distally



where the second portion of the duodenum was seen to be normal. In the small bowel, typical changes of regional ileitis were demonstrated over approximately 3 feet of distal ileum. At least 3 skip lesions were identified which showed considerable narrowing, irregularity of the mucosa, and small nodular lucencies suggesting heaped up mucosa (Fig. 3). Radiographic studies of the colon, urinary tracts, gall bladder, and chest revealed no abnormality.



Case 256, Fig. 1. Anteroposterior view of the stomach and duodenum shows a normal proximal stomach but no clear demarcation between the antrum and duodenum. The duodenal bulb and first portion are narrowed. No mucosal folds are present. A sharp transition occurs at the junction with the proximal second portion of the duodenum.

The patient had no symptoms of obstruction and there was no bleeding. He was treated symptomatically. Abdominal pain disappeared and diarrhea abated. He was asymptomatic on the eighth hospital day and was discharged improved.

#### DISCUSSION

Although granulomatous disease of the duodenum has been described many times, it is rare to demonstrate the classical radiographic picture that we have come to expect with granulomatous disease of the more distal small bowel. Some

of the cases described in the literature show what may be interpreted as secondary involvement of the duodenum due to granulomatous reaction in the adjacent mesentery and retroperitoneal structures. In the case presented we have an exquisite demonstration of segmental involvement of the duodenal bulb.

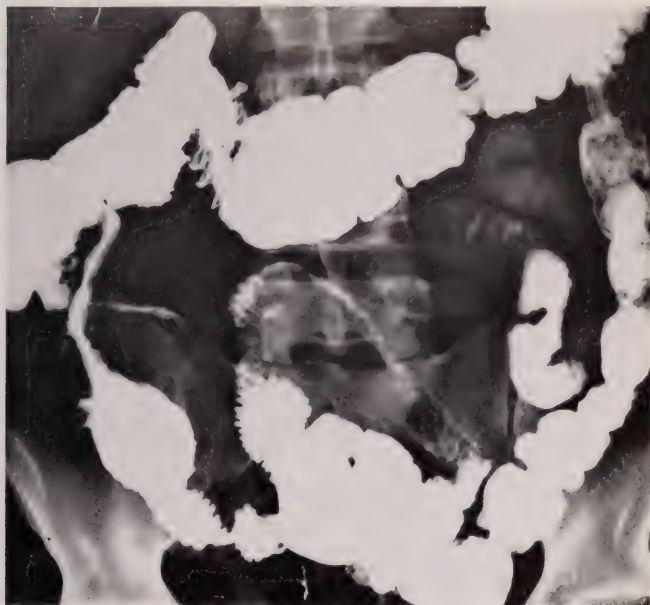


Case 256, Fig. 2. Spot view of the antrum and duodenum show a frayed mucosal surface. There are no normal folds. The segment has distended somewhat in comparison with Fig. 1.

clearly an intrinsic lesion. The segment is narrowed and the mucosa is frayed with no normal mucosal folds present. A sharp transition to normal bowel occurs just as is seen in the ileum. The correct diagnosis is established in this case by the association with classical regional ileitis. The differential diagnosis of the duodenal bulb lesion itself should include Hodgkin's disease which can narrow the bowel as well as destroy the normal mucosa. Boeck's sarcoid would be a rare theoretical possibility. Peptic ulcer disease should be excluded by the

absence of a crater, the complete loss of mucosal folds, the relatively long tapered narrow segment, as well as the lack of any associated functional changes.

*Case Report:* REGIONAL ENTERITIS OF THE DUODENUM.



Case 256, Fig. 3. Anteroposterior view of the abdomen 3 hours after ingestion of barium reveals multiple skipped narrowed segments typical of regional enteritis. The normal mucosal pattern is completely absent and areas of cobble-stone pattern can be seen. Intra-mural sinus tracts are seen along the margins of the terminal ileum.

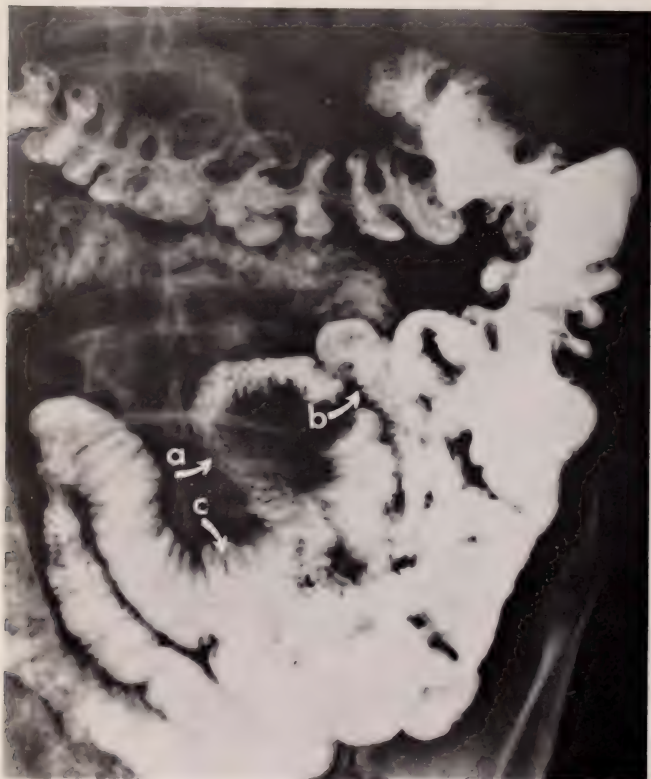
#### ACKNOWLEDGMENT

This case is presented through the courtesy of Dr. Sam Alhadeff, Good Samaritan Hospital, Suffern, N. Y.

#### CASE NO. 257

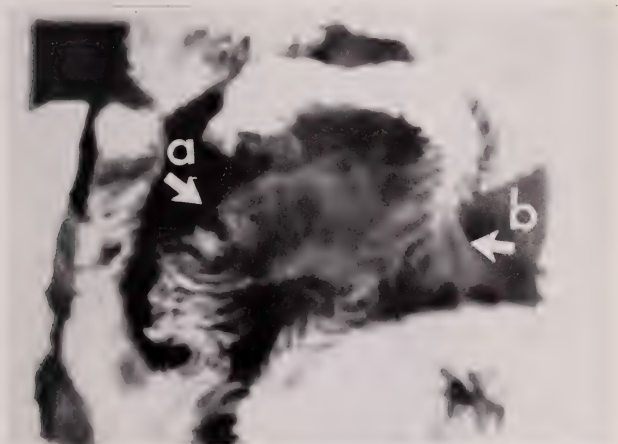
A 64 year old male was admitted to the hospital because of right lower quadrant abdominal pain, nausea and vomiting. Physical examination revealed marked tenderness in the right lower quadrant and signs of peritoneal irrita-

tion. There was elevation of the white blood count with many young forms and a mild temperature elevation. Clinical impression was acute appendicitis. At exploration the appendix was indeed acutely inflamed and appendectomy was performed. In addition, however, a large mass was encountered involving small



Case 257, Fig. 1. Anteroposterior film of the abdomen (reversed in reproduction) 90 minutes after ingestion of barium reveals a hoop shaped loop which maintained its configuration on many views including pressure studies. The mucosal pattern is shirred and fixed (arrow a) in one limb of the loop. The opposite limb contains a short narrowed segment (covered by arrow b) with smoothly shouldered margins. Arrow c points in the direction of the entero-enterostomy.

bowel and mesenteric nodes. A small nodular lesion of the mid-ileum was located. The mesentery was infiltrated and local and regional lymph nodes were greatly enlarged. Several metastatic nodules were seen in the liver. The nodular lesion had narrowed the lumen and the proximal bowel was moderately dilated. A biopsy was performed and an entero-enterostomy was created to bypass the lesion. The pathologist reported carcinoid tumor with infiltration of the wall of the bowel. The appendix revealed acute suppurative inflammation but no evidence of carcinoid tumor.



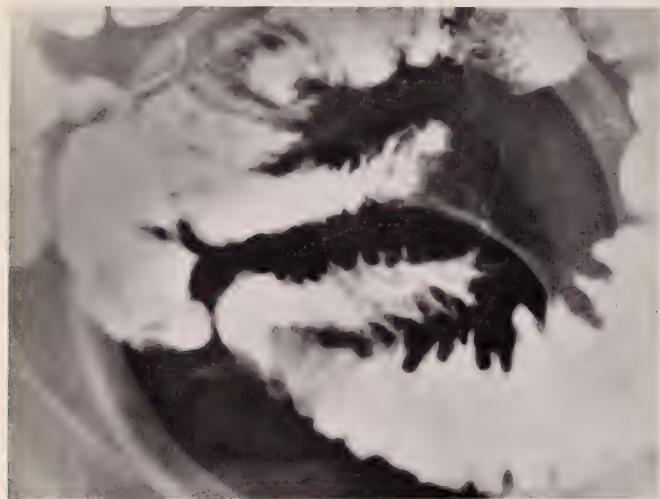
Case 257, Fig. 2. Spot pressure study (conventional orientation) of the abnormal loop shows fixed, shirred folds below the narrowed segment (arrow a) and also in the opposite limb (arrow b).

The patient made an uneventful recovery and was discharged from the hospital. He remained asymptomatic for three months. There was no clinical evidence of carcinoid syndrome. He gradually developed abdominal distention, nausea, vomiting and right lower quadrant abdominal pain. A large mass rapidly developed in the right lower quadrant. A course of chemotherapy was administered combining Thio-tepa and Methotrexate with marked decrease in the size of the mass and almost complete disappearance of symptoms. He remained improved for three months when symptoms recurred and the mass again increased in size.

The patient was admitted to the hospital and radiographic study of the gastrointestinal tract was performed (Figs. 1 and 2). The proximal small bowel was not dilated. In the right lower quadrant a hoop-shaped loop of ileum was seen which maintained a fixed position. In one limb of this loop there was a short

eccentrically narrowed segment with smooth shouldered margins proximally and distally. The mucosal pattern in the opposite limb was shirred and fixed. The entero-enterostomy was located. The short narrowed segment was felt to contain the primary tumor nodule and the fixed loop and distorted mucosa to be evidence of local metastatic spread.

A course of 5-fluoro uracil was administered. Again the mass shrunk remarkably and symptoms virtually disappeared. At present the patient is being main-



Case 257, Fig. 3. Another proven case of carcinoid tumor involving distal ileum showing a sharply angulated and fixed loop with thickened, pleated fold pattern. The apex of the loop is narrowed over a wide area. The proximal bowel is dilated.

tained on intermittent doses of 5-fluoro uracil. The mass in the right lower quadrant is barely palpable and he has only an occasional episode of pain. Sixteen months have now elapsed since the original diagnosis. There continues to be no clinical evidence of carcinoid syndrome.

#### DISCUSSION

Carcinoid tumor of the small bowel is not a rare entity, but it is distinctly rare to demonstrate the primary tumor. These tumors are usually small and originate in an extra-mucosal location. They spread characteristically via the lymphatics into the adjacent mesentery and encourage a marked scirrhus reac-



tion. This results in angulation and fixation of the adjacent bowel loops. Figures 1 and 2 demonstrate the short eccentrically narrowed segment containing the primary tumor nodule. The fixed hoop-shaped loop with distorted mucosal folds is evidence of the associated scirrhus reaction in the mesentery. These changes are characteristic of carcinoid tumor and the angulation of the bowel is often even more marked and acute (Fig. 3). Differential diagnosis should include metastatic carcinoma to the small bowel and also primary carcinoma of the small bowel with local metastases, both of which can produce an identical picture.

*Case Report:* CARCINOID TUMOR OF THE SMALL BOWEL (two cases).

#### ACKNOWLEDGMENT

This case is presented through the courtesy of Dr. Elias Tsoukas, Good Samaritan Hospital, Suffern, N.Y.



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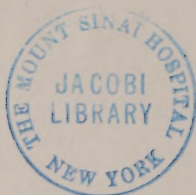
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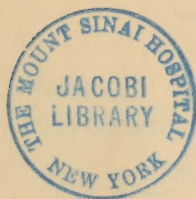






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